[18F](2S,4S)-4-(3-Fluoropropyl)glutamine as a Tumor Imaging Agent

Zehui Wu,‡,§ Zhihao Zha,‡,§ Genxun Li,† Brian P. Lieberman,‡ Seok Rye Choi,‡ Karl Ploessl,† and Hank F. Kung*†,‡

†Departments of Radiology and ‡Pharmacology, University of Pennsylvania, 3700 Market Street, Philadelphia, Pennsylvania 19104, United States

ABSTRACT: Although the growth and proliferation of most tumors is fueled by glucose, some tumors are more likely to metabolize glutamine. In particular, tumor cells with the upregulated c-Myc gene are generally reprogrammed to utilize glutamine. We have developed new 3-fluoropropyl analogs of glutamine, namely [18F](2S,4R)- and [18F](2S,4S)-4-(3-fluoropropyl)glutamine, 3 and 4, to be used as probes for studying glutamine metabolism in these tumor cells. Optically pure isomers labeled with 18F and 19F (2S,4S) and (2S,4R)-4-(3-fluoropropyl)glutamine were synthesized via different routes and isolated in high radiochemical purity (≥95%). Cell uptake studies of both isomers showed that they were taken up efficiently by 9L tumor cells with a steady increase over a time frame of 120 min. At 120 min, their uptake was approximately two times higher than that of L-[3H]glutamine ([3H]Gln). These in vitro cell uptake studies suggested that the new probes are potential tumor imaging agents. Yet, the lower chemical yield of the precursor for 3, as well as the low radiochemical yield for 3, limits the availability of [18F](2S,4R)-4-(3-fluoropropyl)glutamine, 3. We, therefore, focused on [18F](2S,4S)-4-(3-fluoropropyl)glutamine, 4. The in vitro cell uptake studies suggested that the new probe, [18F](2S,4S)-4-(3-fluoropropyl)glutamine, 4, is most sensitive to the LAT transport system, followed by System N and ASC transporters. A dual-isotope experiment using L-[3H]glutamine and the new probe showed that the uptake of [3H]Gln into 9L cells was highly associated with macromolecules (>90%), whereas the [18F](2S,4S)-4-(3-fluoropropyl)glutamine, 4, was not (<10%). This suggests a different mechanism of retention. In vivo PET imaging studies demonstrated tumor-specific uptake in rats bearing 9L xenographs with an excellent tumor to muscle ratio (maximum of ~8 at 40 min). [18F](2S,4S)-4-(3-fluoropropyl)glutamine, 4, may be useful for testing tumors that may metabolize glutamine related amino acids.

KEYWORDS: cancer, metabolism, PET imaging, glutamine and radiolabeling

INTRODUCTION

In the past two decades, the use of 2-[18F]fluoro-2-deoxy-D-glucose (FDG) and positron emission tomography (PET) has achieved widespread acceptance as an effective tool for detecting cancers with high rates of glycolysis. It is generally accepted that a high rate of glucose metabolism (Warburg effect) is associated with changes in tumor-driven alternative gene expression.1,2 However, despite the tremendous promise of FDG-PET for detecting and monitoring tumor metabolism, a significant portion of malignant tumors are not FDG-positive and can be missed in a FDG-PET scan. Accordingly, there is a clear and urgent need to develop additional metabolic tracers, particularly for cancers with low FDG-uptake.

Recent reports suggest that metabolic reprogramming may cause some cancers to switch their energy source from glucose to glutamine.3-6 These tumors could be imaged with [18F]-labeled glutamine tracers.7-9 Glutamine, which is found circulating in the blood and is also concentrated in the skeletal muscles (0.5–1 mmol/L), has various critical functions: as a substrate for DNA and protein synthesis, a primary source of fuel for cells lining the inside of the small intestine and rapidly dividing immune cells, and as a regulator of acid–base balance by producing ammonium in the kidneys. Enhanced glutamine utilization in cancers due to changes in the expression of oncogenic signaling pathways can lead to glutaminolysis. In these cases, blocking glutamine synthetic pathways may lead to tumor cell death.10 Glutamine imaging agents may be useful for testing the therapeutic efficacy of antitumor agents aimed at reducing glutamine metabolism in tumors.

In order to study glutamine metabolizing tumors, we previously prepared and tested L-5-[13C]glutamine.7 In tumor cell uptake studies, the maximum uptake of L-5-[13C]glutamine reached 17.9 and 22.5% uptake/100 μg protein at 60 min in 9L and SF188 tumor cells, respectively. At 30 min after incubation, >30% of the activity appeared to be incorporated into cellular proteins. Dynamic small animal PET studies in rats bearing xenographs of 9L tumor and in transgenic mice bearing...
spontaneous mammary gland tumors showed prominent tumor uptake and retention. The results suggested that L-S-[11C]glutamine would be useful for probing in vivo tumor metabolism in glutaminolytic tumors.

Because [11C] has a half-life of 20 min, it would not be practical for most clinical settings. To make an imaging agent that would be better for clinical use, we created an alternative metabolic tracer labeled with [18F], which has a half-life of 110 min. In vitro studies with [18F](2S,4R)-4-fluoroglutamine, 2 ([18F](2S,4R)-4-FGln) showed that both 9L and SF188 tumor cells displayed a high rate of glutamine uptake (maximum uptake≈16% dose/100 µg protein), and the radioactivity trapped inside the cell was associated with the macromolecular fraction precipitated by trichloroacetic acid (TCA). The cell uptake of [18F](2S,4R)-4-FGln, 2, by SF188 cells is comparable to that of [11C]-glutamine but higher than that of FDG. Biodistribution and PET imaging studies showed that [18F](2S,4R)-4-FGln, 2, localized in tumors with a higher uptake than that of surrounding muscle and liver tissues, suggesting that [18F]-(2S,4R)-4-FGln, 2, is selectively taken up and trapped by the tumor cells.

One of the drawbacks of [18F](2S,4R)-4-FGln, 2, (and its related optical isomers) is the radio labeling reaction, which is relatively difficult and prone to formation of stereoisomers due to a secondary fluorination reaction. To avoid this complication, we have designed and tested [18F](2S,4R)-4-(3-fluoropropyl)glutamines, 3, ([18F](2S,4R)-4-FPGln), and [18F](2S,4S)-4-(3-fluoropropyl)glutamines, 4, ([18F](2S,4S)-4-FPGln), as alternative probes for imaging glutamine metabolism (Figure 1).

The 4-(3-fluoropropyl)glutamates contain an extended propyl group. This will make it easier for the SN2 group. This will make it easier for the SN2 reaction. To preserve the amide functional group at the C5 position, we have synthesized two types of precursors suitable for radio labeling (fluoro for toslyate substitution reaction). Reported herein is the preparation and in vitro and in vivo studies of these glutamine analogs.

### EXPERIMENTAL SECTION

#### General Information

All reagents used were commercial products and were used without further purification unless otherwise indicated. Boc-Glu(OBzl)–OH (Boc-L-glutamic acid 5-benzyl ester, 15) was purchased from Sigma-Aldrich. Flash chromatography (FC) was performed using silica gel 60 (230–400 mesh, Sigma-Aldrich). 1H NMR spectra were obtained at 200 MHz and 13C NMR spectra were recorded at 50 MHz (Bruker DPX 200 spectrometer). Chemical shifts are reported as δ values (parts per million) relative to remaining protons in deuterated solvent. Coupling constants are reported in hertz. The multiplicity is denoted by s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), br (broad), or m (multiplet). HPLC analyses were performed on an Agilent LC 1100 series. High-resolution MS experiments were performed using an Agilent Technologies LC/MSD TOF mass spectrometer.

#### Syntheses

Compounds 5–8 were synthesized according to the procedures reported previously.\(^9\)

(5)-tert-Butyl 2-(tert-butoxy carbonylamino)-4-(tosyloxy) Butanoate (9). To a solution of compound 8 (544 mg, 2 mmol) in 10 mL of dry dichloromethane (DCM) was added Et3N (1.4 mL, 10 mmol), 4-(dimethylamino)pyridine (DMAP, 24 mg, 0.2 mmol), and p-toluenesulfonyl chloride (TsCl, 764 mg, 4 mmol) at 0 °C. The mixture was stirred at room temperature (rt) overnight. Ice-cold water (15 mL) was poured into the reaction mixture and the mixture was extracted with DCM (15 × 3 mL), the combined organic layer was dried with MgSO4 and was purified with flash chromatography (FC, EtOAc/hexane = 2/8) to give 739.6 mg (yield: 86.1%). 1H NMR (200 MHz, CDCl3) δ: 1.39–1.54 (m, 18H), 1.90–2.05 (m, 2H), 2.47 (s, 3H), 4.06–4.09 (m, 2H), 4.13–4.16 (m, 1H), 5.03–5.10 (m, 1H), 7.37 (d, J = 7.8 Hz, 2H), 7.81 (d, J = 8.2 Hz, 2H). HRMS was calcld for C18H19NO5S (M + H)+: 430.1899. Found: 430.1910.

(5)-tert-Butyl 2-(tert-butoxycarbonylamino)-4-cyanobutanoate (10). Sodium iodide (240 mg, 1.6 mmol) was added to a solution of compound 9 (343 mg, 0.8 mmol) in 10 mL of acetone (HPLC grade) at rt. The mixture was stirred at 60 °C for 3 h. The solvent was then removed and the residue was dissolved in 15 mL of DCM. The precipitated solid was filtered out and filtrate was concentrated. N,N-Dimethylformamide (DMF, 7 mL) and potassium cyanide (78 mg, 1.2 mmol) were added to the residue. The mixture was stirred at rt overnight. The reaction mixture was quenched with 25 mL of ethyl acetate and was washed with H2O (10 × 3 mL). The organic layer was dried over MgSO4 and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 2/8) to give 196.2 mg colorless oil (yield: 86.3%). 1H NMR (200 MHz, CDCl3) δ: 1.39–1.52 (m, 18H), 1.90–2.04 (m, 1H), 2.20–2.30 (m, 1H), 2.40–2.46 (m, 2H), 4.20–4.30 (m, 1H), 5.12–5.22 (m, 1H). HRMS was calcld for C16H19NO4 (M + H)+: 320.2080. Found: 320.2078.

(2S,4S)-tert-Butyl 2-(tert-butoxycarbonylamino)-4-cyano-hept-6-enoate (11a) and (2S,4R)-tert-Butyl 2-(tert-butoxy-
carbonylamino)-4-cyanohept-6-enoate (11b). LiHMDS (lithium bis(trimethylsilyl)amide) (1.5 mL, 1 mol/L solution in THF) was added to a three-necked 250 mL flask. The mixture was cooled down to −78 °C with a dry ice–acetone bath. Compound 10 (201 mg, 0.67 mmol) in 3 mL of dry THF solution was added dropwise over 30 min. After being stirred at −78 °C for 2 h, allyl bromide (0.24 mL, 2.8 mmol) was added dropwise over 15 min. The mixture was then stirred at −78 °C for another 4 h. The reaction was quenched with 20 mL of ethyl acetate and 15 mL of HCl (2 M) and extracted with ethyl acetate and 15 mL of HCl (2 M) and extracted with ethyl acetate (20 × 3 mL). The organic layer was dried over MgSO4 and NaOH (0.9 mL, 1 M), following the same procedure described for compound 13a. Compound 13b was prepared from 12b (102 mg, 0.3 mmol), Et3N (0.21 mL, 1.5 M), TSCI (115 mg, 0.6 mmol) and DMAP (4 mg, 0.03 mmol), following the same procedure described for compound 13a. Compound 13b: 100 mg (yield: 67.1%). 1HNMR (200 MHz, CDCl3) δ: 1.39–1.52 (m, 18H), 1.70–1.90 (m, 4H), 1.91–2.01 (m, 2H), 2.45 (s, 3H), 2.60–2.70 (m, 1H), 3.95–4.15 (m, 2H), 4.16–4.25 (m, 1H), 5.10–5.19 (m, 1H), 7.37 (d, J = 8.0 Hz, 2H). HRMS was calculated for C19H20N2O4S (M + NH4)+: 343.2258. Found: 343.2258.

Compound 13b was prepared from 12b (102 mg, 0.3 mmol), Et3N (0.21 mL, 1.5 M), TSCI (115 mg, 0.6 mmol) and DMAP (4 mg, 0.03 mmol), following the same procedure described for compound 13a. Compound 13b: 100 mg (yield: 67.1%). 1HNMR (200 MHz, CDCl3) δ: 1.39–1.52 (m, 18H), 1.70–1.90 (m, 4H), 1.91–2.01 (m, 1H), 2.15–2.30 (m, 1H), 2.50 (s, 3H), 2.60–2.70 (m, 1H), 3.95–4.15 (m, 2H), 4.20–4.35 (m, 1H), 5.10–5.19 (m, 1H), 7.37 (d, J = 8.2 Hz, 2H). HRMS was calculated for C25H31N4O-S (M + NH4)+: 514.2587. Found: 514.2589.

(25,45)-tert-Butyl 2-(tert-Butoxy carbonylamino)-4-cyano-7-fluoroheptanoate (14a) and (25,45)-tert-Butyl 2-(tert-Butoxy carbonylamino)-4-cyano-7-fluoroheptanoate (14b). To a solution of tris(dimethylamino)sulfonium di trifluoromethanesulfonate (TASF, 100 mg, 0.363 mmol) in 3 mL of DCM and 3 mL of DMF was added Et3N(HF)3 (0.021 mL) dropwise followed by 13a (35 mg, 0.072 mmol) in 3 mL of DCM. The mixture was then heated at 50 °C overnight. The reaction was quenched by the addition of ice-cold water (5 mL) and diluted with 50 mL of EtOAc, and then washed with H2O (15 mL × 2) and brine (15 mL) and dried with MgSO4. The filtrate was evaporated in vacuo and the residue was purified by FC (EtOAc/hexane = 2/8) to give 22 mg of colorless oil 14a (yield: 88.8%). 1HNMR (200 MHz, CDCl3) δ: 1.39–1.52 (m, 18H), 1.70–1.90 (m, 2H), 2.05–2.25 (m, 1H), 2.3–2.55 (m, 2H), 2.71–2.85 (m, 1H), 3.45–4.45 (m, 1H), 5.22–5.36 (m, 3H), 5.70–5.91 (m, 1H). HRMS was calculated for C25H31N4O-S (M + NH4)+: 343.2257. Found: 343.2252.

(25,45)-tert-Butyl 2-(tert-Butoxy carbonylamino)-4-cyano-7-hydroxy-heptanoate (12a) and (25,4R,4R)-tert-Butyl 2-(tert-Butoxy carbonylamino)-4-cyano-7-hydroxyheptanoate (12b). To a solution of compound 11a (91 mg, 0.28 mmol) in 7 mL of THF was added 9-borabicyclo[3.3.1]nonane (9-BBN, 2.22 mL, 0.5 M solution in THF) dropwise at 0 °C. After being stirred at 0 °C for 1 h, the reaction mixture was moved to rt and stirred for another 48 h. The mixture was then cooled with an ice-bath. H2O (0.31 mL of 30 wt % solution in H2O) and NaOH (0.4 mL, 1 M) were added dropwise. The mixture was stirred at rt for 30 min, diluted with 15 mL of H2O and extracted with ethyl acetate. The organic layer was dried over MgSO4 and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 1/1) to give 30.7 mg colorless oil 12a (yield: 32.1%). 1HNMR (200 MHz, CDCl3) δ: 1.39–1.52 (m, 18H), 1.80–1.95 (m, 4H), 1.96–2.15 (m, 2H), 2.70–2.85 (m, 1H), 3.69–3.75 (m, 2H), 4.20–4.35 (m, 1H), 5.20–5.35 (m, 1H). HRMS was calculated for C19H20N2O4 (M + H)+: 325.2127. Found: 325.2125.

Compound 12b was prepared from 11b (194 mg, 0.66 mmol), 9-BBN (4.8 mL, 0.5 M solution in THF), H2O (0.65 mL of 30 wt % solution in H2O), and NaOH (0.9 mL, 1 M), following the same procedure described for compound 12a. Compound 12b: 91 mg (yield: 44.6%). 1HNMR (200 MHz, CDCl3) δ: 1.39–1.52 (m, 18H), 1.70–1.85 (m, 4H), 1.86–2.01 (m, 1H), 2.09–2.30 (m, 1H), 2.70–2.85 (m, 1H), 3.69–3.75 (m, 2H), 4.25–4.40 (m, 1H), 5.10–5.25 (m, 1H). HRMS was calculated for C25H31N4O4 (M + H)+: 343.2233. Found: 343.2238.
(m, 1H), 4.41 (dt, J = 47.4 Hz, J = 5.0 Hz, 2H). 13CNMR (50 MHz, D2O): δ 179.9, 174.0, 84.82 (d, J = 157.5 Hz), 53.0, 42.1, 33.4, 27.4 (d, J = 30 Hz, 27.4). HRMS was calcld for C21H32NO6 (M + NH4)+: 397.2331. Found: 397.2347.

(S)-5-Benzyl 1-tert-Butyl 2-(tert-Butoxy carbonylamino)-pentanedioate (16). To a solution of Boc-Glu(Obzl) (18 mg, 0.05 mmol) in 17 mL of DMF and 2 mL of DMF was added Et3N (1.9 mmol, 1.26 mL) and stirred for another 2 h. Allyl bromide (2.4 g, 20 mmol, 1.27 mL) was added through a syringe. The mixture was then stirred for another 48 h. The mixture was filtered and the filtrate was concentrated to 700 mg of colorless oil 17 (yield: 77.4%). 1HNMR (200 MHz, CDCl3) δ: 1.43–1.45 (m, 18H), 1.70–1.73 (m, 4H), 1.86–1.89 (m, 2H), 2.49–2.63 (m, 1H), 2.93–3.08 (m, 1H), 3.40–3.55 (m, 2H), 4.24–4.36 (m, 1H), 5.09–5.12 (m, 3H), 7.36 (s, 5H). HRMS was calcld for C21H32NO6 (M + H)+: 397.2331. Found: 397.2347.

To a solution of compound 17 (860 mg, 2 mmol) in 7 mL of THF was added 9-BBN (8 mL, 0.5 M solution in THF) dropwise at 0 °C. After stirring at 0 °C for 1 h, the reaction mixture was neutralized with 1N aq. HCl solution and filtered. The filtrate was concentrated to give 700 mg of colorless oil 18 (yield: 77.4%). 1HNMR (200 MHz, CDCl3) δ: 1.43–1.45 (m, 18H), 1.70–1.73 (m, 4H), 1.86–1.93 (m, 2H), 2.49–2.63 (m, 1H), 2.93–3.08 (m, 1H), 3.40–3.55 (m, 2H), 4.24–4.36 (m, 1H), 5.09–5.12 (m, 3H), 7.36 (s, 5H). HRMS was calcld for C21H32NO6 (M + H)+: 397.2331. Found: 397.2347.

(2S,4S)-1-Benzyl 5-tert-Butyl 4-(tert-Butoxycarbonylamino)-5-oxo-2-((3-tetrahydro-2H-pyran-2-yl)oxy)propylpentanedioate (20). A mixture of the ester 19 (750 mg, 1.4 mmol) and 10% Pd/C (90 mg) in absolute EtOH (15 mL) was stirred under hydrogen overnight. This mixture was then filtered and the filtrate was concentrated to give 620 mg of colorless oil 20 (yield: 92%). 1HNMR (200 MHz, CDCl3) δ: 1.45–1.47 (m, 18H), 1.50–1.70 (m, 6H), 1.75–1.80 (m, 2H), 1.82–2.00 (m, 4H), 2.51–2.58 (m, 1H), 3.41–3.51 (m, 2H), 3.80–3.95 (m, 2H), 4.22–4.29 (m, 1H), 4.59 (s, 1H), 4.05–5.01 (m, 1H). HRMS was calcld for C23H34NO8 (M + H)+: 446.2754. Found: 446.2740.

(2S,4S)-1-Benzyl 5-tert-Butyl 2-((tert-Butoxycarbonylamino)-5-oxo-2-((3-tetrahydro-2H-pyran-2-yl)oxy)propyl)pentanedioate (21). To a solution of compound 20 (534 mg, 1.2 mmol) in 2 mL of DCM and 2 mL of DMF was added Et3N (1.9 mmol, 0.26 mL) and HOBr (1.44 mmol, 297 mg), 2,4,6-trimethoxybenzylamine hydrochloride (370 mg, 1.58 mmol), and N,N-dicyclohexylcarbodiimide (276 mg, 1.8 mmol) at 0 °C. The mixture was stirred for 24 h. A total of 30 mL of EtOAc was added to the reaction mixture. The mixture was then washed with brine (10% in H2O, 5 mL), H2O (5 mL × 2) as well as brine (5 mL), dried over Na2SO4, and filtered. The filtrate was concentrated to give 624 mg of oil. The residue was dissolved in 4 mL of EtOAc. Pyridinium p-toluenesulfonate (50 mg, 0.2 mmol) was then added. After heating at 50 °C for 4 h, the solvent was evaporated in vacuo and the residue was purified by FC (EtOAc/hexanes = 1/1) to give 203 mg of colorless oil 21 (yield: 30.8%). 1HNMR (200 MHz, CDCl3) δ: 1.44–1.45 (m, 18H), 1.46–1.70 (m, 4H), 1.81–1.86 (m, 2H), 2.14–2.21 (m, 2H), 3.54–3.60 (m, 2H), 3.82 (s, 9H), 4.12–4.18 (m, 1H), 4.28–4.37 (m, 1H), 4.53–4.62 (m, 1H), 5.04–5.09 (m, 1H), 5.95 (s, 1H), 6.13 (s, 1H). HRMS was calcld for C23H34NO8 (M + H)+: 446.2754. Found: 446.2740.
The radiosynthesis was performed by a similar method as described previously. Briefly, an activated SepPak Light QMA Carb was loaded with $^{[18]F}$fluoride (740 to 1480 MBq (20 to 40 mCi)) and eluted with 1 mL of 18-crown-6/KHCO$_3$ (160 mg of 18-crown-6 in 18.6 mL of ACN/29 mg of KHCO$_3$ in 3.4 mL of water). The solution was blown with argon until dry and dried twice azeotropically with 1 mL of acetonitrile at 80 °C under a flow of argon. The dried $^{[18]F}$fluoride was cooled in an ice bath and 5 mg of tosylate precursor (O-tosylate, 13a and 13b, respectively) was dissolved in 0.5 mL of DMSO and added to the dried $^{[18]F}$fluoride. The mixture was heated for 10 min at 110 °C in an oil bath. The mixture was then cooled in an ice bath and added to 6 mL of water/1 mL of acetonitrile. The mixture was loaded onto an activated Oasis HLB 3 cm$^3$ cartridge, pushed through, and washed twice with 3 mL of water. The desired radiolabeled compound was eluted with 1 mL of acetonitrile. The acetonitrile solution was blown until dry. A mixture of 400 μL of TFA/100 μL of $\text{H}_2\text{SO}_4$ was added and heated for 10 min at 120 °C in a capped 10 mL vial. TFA was removed under argon while still warm. The reaction tube was then cooled in an ice bath. Water (1 mL) was slowly added and the mixture was neutralized by the slow addition of a saturated Na$_2$CO$_3$ solution under heavy shaking (∼1000 μL) (pH ∼8). The mixture was put through an activated Oasis HLB 3 cm$^3$, which was topped with ∼0.3 g of Ag$_{11}$A8 resin. The radioactivity was eluted with phosphate buffered saline (pH 7.0) in fractions of 0.5 mL volume to yield the desired radioactive $^{[18]F}$[25,45]-4-FPGln, $^{[3]F}$-4-FPGln, and $^{[18]F}$-25,45)-4-FPGln, respectively.

The radiochemical and stoicheiometric purities were determined by two different HPLC systems. System 1. Column: Gemini 3u C18 150 × 4.6 mm, 3 μm. Mobile phase (gradient) Solvent A: ACN. Solvent B: 0.1% FA. Gradient, 1 mL/min: 0–3 min 95% B, 3–11 min 95%–5% B, 11–19 min 5%–95% B, 19–21 min 95% B. Retention times for both 3 and 4 are ∼2.5 min. System 2. Column: Chirex 3126 (ι)-penicillamine 250 × 4.6 mm, 4.6 μm. Mobile phase (isocratic): 2 mM Cu$_2$SO$_4$ solution, 1 mL/min, column temperature at 30 °C. The retention times of 3 and 4 are 14.6 and 20 min, respectively.

Alternatively, TmobNH precursor, 22, was used under a similar labeling condition as described above (18-crown-6/ KHCO$_3$/ACN/80 °C/20 min). It was found that the TmobNH intermediate, 26, was formed with a lower yield of 6.6 ± 1.6%, radiochemical purity 98% ($n = 3$). The $^{[18]F}$ intermediate, $^{[18]F}$26, displayed the same profile on the HPLC as that of the “cold” compound. Deprotection was performed with 500 μL of TFA at 40 °C for 8 min. Volatiles were removed under argon while still warm. The residue was treated with 1 mL of phosphate buffered saline (PBS) and filtered through a 0.45 μm filter and washed with 0.1 mL of PBS (pH 7.0) to give a crude dose. The solution was passed through an activated cartridge (Oasis HLB 3 cm$^3$). The solid-phase extraction was further rinsed with 0.3 mL of PBS (pH 7.0) to yield (25,45)$^{[18]F}$-4-FPGln, 4.

**Cell Uptake Studies.** 9L cells (ATCC, Manassas, VA) were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM, GibCO BRL, Grand Island, NY) supplemented with 10% fetal bovine serum (HyClone, Logan, UT) and 1% 100 units/mL penicillin, 100 μg/mL streptomycin. The cells were maintained in T-75 culture flasks under humidified incubator conditions (37 °C, 5% CO$_2$) and were routinely passaged at confluence.
Tumor cells were plated in 12 well plates 24 h prior to studies. On the day of the experiment, the culture media was aspirated and the cells were washed three times with warm PBS (containing 0.90 mM of Ca$^{2+}$ and 1.05 mM of Mg$^{2+}$). $^{[18F]}(2\text{S},4\text{R})$-4-FPGln, 3 (370 kBq) and L-$^{[3,4-3\text{H}(\text{N})]}$-glutamine ($^{[3\text{H}]}$Gln) (37 kBq) were mixed in PBS (with Ca$^{2+}$ and Mg$^{2+}$) solution and then added to each well. The same procedure was performed with $^{[18F]}(2\text{S},4\text{S})$-4-FPGln, 4, and $^{[3\text{H}]}$Gln. The cells were incubated at 37 $^\circ$C for 5, 30, 60, and 120 min. At the end of the incubation period, the PBS solution containing the ligands was aspirated and the cells were washed three times with 1 mL of ice cold PBS (without Ca$^{2+}$ and Mg$^{2+}$). After washing with ice-cold PBS, 350 μL of 0.1 N NaOH was used to lyse the cells. The lysed cells were collected onto filter paper and counted together with samples of the incubation dose using a gamma counter (Packard Cobra). After 24 h, $^3$H activity was counted using a scintillation counter (Beckman LS 6500). A total of 100 μL of the cell lysate was used to determine the protein concentration (Modified Lowry Protein Assay). The data was normalized as percentage uptake of initial dose (ID) relative to 100 μg of protein content (% ID/100 μg of protein).

Scheme 1. Production of $^{[18F]}(2\text{S},4\text{R})$-4-(3-fluoropropyl)glutamine

| Step | Reagents and reaction conditions: |
|------|----------------------------------|
| a    | tert-butyl 2,2,2-trichloroacetimidate (TBTBA), BF$_3$Et$_2$O, DCM, cyclohexane, rt, overnight |
| b    | Pd/C, H$_2$, MeOH, rt, overnight |
| c    | ECF, NaBH$_4$, THF, H$_2$O, $-15$–$-0$ $^\circ$C, 4 h |
| d    | TsCl, Et$_3$N, DMAP, DCM, rt, overnight |
| e    | NaI, KCN, DMF, rt, overnight |
| f    | LiHMDS, Allyl bromide, THF, $-78$ $^\circ$C, 4 h |
| g    | 9-BBN, H$_2$O$_2$, NaOH, 0 $^\circ$C - rt, 48 h |
| h    | TASF, Et$_3$N(HF)$_3$, DCM, DMF, 50 $^\circ$C, overnight |
| i    | HCl, rt, 6 h |

Protein Incorporation of $^{[18F]}(2\text{S},4\text{S})$-4-FPGln, 4, into 9L Tumor Cells. To test the in vivo cell incorporation, we used 9L cells. Cells were plated (5 × 10$^5$ cells/well) on six-well plates in culture media 24 h prior to the experiment. On the day of experiment, the media was aspirated and the cells were washed three times with 4 mL of warm PBS (containing 0.90 mM of Ca$^{2+}$ and 1.05 mM of Mg$^{2+}$). To measure the extent of protein incorporation of $^{[18F]}(2\text{S},4\text{S})$-4-FPGln, 4, protein bound activity in 9L cells was determined at 30 and 120 min after incubation. $^{[18F]}(2\text{S},4\text{S})$-4-FPGln, 4 (370 kBq) and L-$^{[3,4-3\text{H}(\text{N})]}$-glutamine ($^{[3\text{H}]}$Gln, 37 kBq) in 2 mL of PBS were mixed in the incubation media.

Tumor cells were plated in 12 well plates 24 h prior to studies. On the day of the experiment, the culture media was aspirated and the cells were washed three times with warm PBS (containing 0.90 mM of Ca$^{2+}$ and 1.05 mM of Mg$^{2+}$). $^{[18F]}(2\text{S},4\text{R})$-4-FPGln, 3 (370 kBq) and L-$^{[3,4-3\text{H}(\text{N})]}$-glutamine ($^{[3\text{H}]}$Gln) (37 kBq) were mixed in PBS (with Ca$^{2+}$ and Mg$^{2+}$) solution and then added to each well. The same procedure was performed with $^{[18F]}(2\text{S},4\text{S})$-4-FPGln, 4, and $^{[3\text{H}]}$Gln. The cells were incubated at 37 $^\circ$C for 5, 30, 60, and 120 min. At the end of the incubation period, the PBS solution containing the ligands was aspirated and the cells were washed three times with 1 mL of ice cold PBS (without Ca$^{2+}$ and Mg$^{2+}$). After washing with ice-cold PBS, 350 μL of 0.1 N NaOH was used to lyse the cells. The lysed cells were collected onto filter paper and counted together with samples of the incubation dose using a gamma counter (Packard Cobra). After 24 h, $^3$H activity was counted using a scintillation counter (Beckman LS 6500). A total of 100 μL of the cell lysate was used to determine the protein concentration (Modified Lowry Protein Assay). The data was normalized as percentage uptake of initial dose (ID) relative to 100 μg of protein content (% ID/100 μg of protein).

Protein Incorporation of $^{[18F]}(2\text{S},4\text{S})$-4-FPGln, 4, into 9L Tumor Cells. To test the in vivo cell incorporation, we used 9L cells. Cells were plated (5 × 10$^5$ cells/well) on six-well plates in culture media 24 h prior to the experiment. On the day of experiment, the media was aspirated and the cells were washed three times with 4 mL of warm PBS (containing 0.90 mM of Ca$^{2+}$ and 1.05 mM of Mg$^{2+}$). To measure the extent of protein incorporation of $^{[18F]}(2\text{S},4\text{S})$-4-FPGln, 4, protein bound activity in 9L cells was determined at 30 and 120 min after incubation. $^{[18F]}(2\text{S},4\text{S})$-4-FPGln, 4 (370 kBq) and L-$^{[3,4-3\text{H}(\text{N})]}$-glutamine ($^{[3\text{H}]}$Gln, 37 kBq) in 2 mL of PBS were mixed in the incubation media.
To identify the ligand’s stability in supernatant after precipitation with trichloroacetic acid (TCA), cells were grown in 10 cm dishes and incubated with 1.8 MBq \([^{18}F](2S,4S)-4\text{-}FPGln\), only. At the end of incubation, the radioactive medium was removed, the cells were washed three times with ice cold PBS without Ca\(^{2+}\) and Mg\(^{2+}\), treated with 0.25% trypsin, and resuspended in PBS. The samples were centrifuged (18,000 g, 3 min), the supernatant removed, and the cells were suspended in 200 μL and 1% Triton-X 100 (Sigma, St. Louis, MO). After vortexing, 800 μL of ice cold 15% TCA was added to the solution. After precipitating for 10 min, the cells were centrifuged again (18,000 g, 3 min) and washed twice with 15% ice cold TCA. The radioactivity of both gamma- and beta-emitting isotopes was determined separately for the supernatant and pellet. Protein incorporation was calculated as a percentage of acid precipitable activity.

**In Vitro Transport Characterization Studies (Inhibition Studies).** To characterize the transport of \([^{18}F](2S,4S)-4\text{-}FPGln\), 4, competitive inhibition studies were conducted using the 9L cell line. The tracer was incubated at 37 °C for 30 min. The cells were processed as described above. Various inhibitors were then added to the cells in concentrations ranging from 0.1 to 5 mM in PBS solution. Selected inhibitors included synthetic amino acid transport inhibitors such as N-methyl-α-aminosobutyric acid (MeAIB) for system A, and 2-amino-bicyclo[2.2.1] heptane-2-carboxylic acid (BCH) for system L.\(^{11-13}\) Natural amino acids, such as L-serine and l-glutamine, were also used as inhibitors, although they are not specific for a particular amino acid transport system. The data was compared in reference to uptake of \([^{18}F](2S,4S)-4\text{-}FPGln\), 4, without any inhibitor in PBS solution at pH 7.4.

**Biodistribution Studies in Rats Bearing 9L Tumors.** Studies of the in vivo distribution of \([^{18}F](2S,4S)-4\text{-}FPGln\), 4, were performed in Fischer (F344) rats bearing 9L tumors as reported previously.\(^8\) F344 rats were purchased from Charles River Laboratories (Malvern, PA). 9L tumor cells (~10^6) in PBS (0.2 mL) were injected subcutaneously into the lower right flank of the rat. The tumors took 12–15 days to reach appropriate size (1 cm diameter). All animals were fasted for 12–18 h prior to the study. Six rats per group were used for the biodistribution study. The rats were anesthetized with isoflurane (2–3%) and 0.2 mL of saline solution containing 25 μCi of the ligand was injected intravenously. The rats were sacrificed at 30 and 60 min postinjection by cardiac excision while under isoflurane anesthesia. The organs of interest were removed, weighed, and the radioactivity was counted with a gamma counter (Packard Cobra). The percent dose per gram
was calculated by a comparison of the tissue activity counts to counts of 1% of the initial dose.

**Small Animal Imaging Studies.** Dynamic small animal PET (APET) imaging studies were conducted with [18F]-\((2\,S,4R)-4\)-fluoro-glutamine, \((2\,S,4R)-4\)-FGln, 2, and [\(^{18}\text{F}\)](2\,S,4S)-4-FPGln, 4 similar to that reported previously.\(^8\) All scans were performed on a dedicated animal PET scanner (Mosaic by Phillips) that has a field of view of 11.5 cm. F344 rats with 9L tumors were used for the imaging studies. A total of 22–37 MBq of activity was injected intravenously via the lateral tail vein. All animals were sedated with isoflurane anesthesia (2–3%, 1 L/min oxygen) and were then placed on a heating pad in order to maintain body temperature throughout the procedure. The animals were visually monitored for breathing and any other signs of distress throughout the entire imaging period. The data acquisition began after an intravenous injection of the tracer. All scans were conducted over a period of 120 min (dynamic, 5 min/frame). The frames were reconstructed and then analyzed with AMIDE imaging analysis software.

**RESULTS**

**Synthesis.** In order to produce [\(^{18}\text{F}\)](2\,S,4R)-4-(3-fluoropropyl)glutamine, 3, [\(^{18}\text{F}\)](2\,S,4R)-4-FPGln, 2, and [\(^{18}\text{F}\)](2\,S,4S)-4-(3-fluoropropyl)glutamine, 4 \((\text{[\(^{18}\text{F}\]})(2\,S,4S)-4\)-FPGln\), we employed two different schemes (Schemes 1 and 2) for preparation of nonradioactive “cold” compounds (3 and 4) and the cyanide and -OTs precursors for radiolabeling. One approach was to prepare the corresponding protected 4-cyanide derivatives, which led to the formation of the desired final products. Commercially purchased Boc-Asp(OBzl)-OH, 5, was treated with tert-butyl 2,2,2-trichloroacetimidate/\(\text{BF}_3\cdot\text{Et}_2\text{O}\) at room temperature to give the tert-BuO- ester, 6; the BnO- ester group was converted to the acid, 7, by Pd/C catalyzed hydrogenation. The aspartic acid, 7, was carefully reduced with NaBH\(_4\) in THF/water at –15 to 0 °C to the corresponding alcohol, 8. The alcohol group was successfully converted to the cyamide, 10, through the -OTs intermediate, 9. The cyamide derivative, 10, was treated with LiHMDS and allyl bromide at –78 °C to give (2\,S,4S)-tert-butyl 2-(tert-butoxycarbonylamino)-4-cyanohept-6-enoate (11a) and (2\,S,4R)-tert-butyl 2-(tert-butoxycarbonylamino)-4-cyanohept-6-enoate (11b) (11a to 11b ratio of 2:1 in 28% and 15% isolated yields). A similar reaction was reported previously for preparation of allyl derivatives of aspartate using the dianionic allylation reactions of amino acid derivatives.\(^14\) The allyl derivatives, 11a and 11b, were separated and purified by flash chromatography. They were converted to the corresponding alcohols, 12a and 12b, and following the treatment with tosyl chloride to the O-tosylated 13a and 13b in good yields. The O-tosylated 13a and 13b were treated with TASF, \(\text{Et}_3\text{N}((\text{HF})_3\), DCM, DMF, 50 °C, overnight to give the desired 14a and 14b, in good yields. Optimization of the fluorination reaction condition using TASF and \(\text{Et}_3\text{N}((\text{HF})_3\), as the reagents was reported previously for the preparation of 4-fluoroglutamate.\(^7\) Deprotection using hydrochloric acid at room temperature produced the final end products, [\(^{18}\text{F}\)](2\,S,4R)-4-(3-fluoropropyl)glutamine, 3, and [\(^{18}\text{F}\)](2\,S,4S)-4-(3-fluoropropyl)glutamine, 4. The O-tosylated 13a and 13b were also successfully used for the radiolabeling reaction (see Discussion below).

The second method introduced N-Tmob protected precursors (as a protecting group to preserve the amide) for radiolabeling and deprotection. Previously, we have tested for the preparation of N-Tmob protected precursors for making isomers of 4-fluoroglutamine (4-FGln).\(^5\) We successfully developed \(^{18}\text{F}\) labeling using this precursor under different labeling conditions. We wanted to extend the same method to the synthesis and labeling of [\(^{18}\text{F}\)](2\,S,4S)-4-(3-fluoropropyl)glutamine, 4. To achieve this, we started with commercially available, Boc-Glu(OBzl)–OH, 15. After treating with tert-butyl 2,2,2-trichloroacetimidate/\(\text{BF}_3\cdot\text{Et}_2\text{O}\) at room temperature, it gave the tert-Bu ester, 16. Using the same dianionic allylation reactions of amino acid derivatives, the reaction preferentially produced the protected (2\,S,4S)-4-allyl-glutamate (in 58% yield). It is interesting to note that the reaction led to the (2\,S,4S) isomer only. The allyl group was converted to alcohol, 18, by 9-BBN/\(\text{H}_2\text{O}_2/\text{NaOH}\) in 0 °C. The alcohol was protected by THP, and the O-benzyl ester was hydrolyzed and the acid, 20, was transformed to Tmob-protected amide, 21. The alcohol, 21, was treated with tosyl chloride to the O-tosylated, 22, which is a suitable precursor for a radioactive \(^{18}\text{F}\) labeling reaction. In order to provide an authentic sample, a cold standard, for the first step of the radioactive \(^{18}\text{F}\) labeling reaction, we also prepared compound, 26.

To further confirm the chemical structure, a slow evaporating recrystallization method provided excellent crystals of “cold” (2\,S,4S)-4-FPGln, 4 and the X-ray crystallographic analysis data added support to the structure assignment (Figure 2). The optically pure (2\,S,4S)-4-FPGln, 4, has never been prepared and presented before. In the X-ray crystallographic structures of (2\,S,4R)4-FGln, 2, and (2\,S,4S)-4-FPGln, 4, the amino acid

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**Figure 2.** Comparison of X-ray crystallography structures for (2\,S,4R)-4-fluoro-glutamine, (2\,S,4R)-4-FGln, 2, and (2\,S,4S)-4-(3-fluoropropyl)-glutamine, (2\,S,4S)-4-FPGln, 4. ORTEP drawing of the title compounds were shown with 30% probability thermal ellipsoids. (Crystal structure, (2\,S,4S)-4-FPGln, 4, was submitted to the Cambridge Crystallographic Data Centre (CCDC 991692).
groups on the right side of the molecules were comparable, whereas the amide group on the left appeared to be varied and different from each other. The results reported in Figure 2 firmly establish the conformation, which may facilitate future use of other 4-fluoroglutamine isomers for biological and medical applications.

Radiolabeling for \([^{18}F](2S,4R)-4-(3\text{-fluoropropyl})\text{-glutamine}, 3 \) and \([^{18}F](2S,4S)-4-(3\text{-fluoropropyl})\text{-glutamine}, 4\). Radiolabeling of the desired \([^{18}F](2S,4R)-4-FPGln, 3\) and \([^{18}F](2S,4S)-4-FPGln, 4\) was achieved by methods in Scheme 3. The preparation can be accomplished by using the O-tosylated cyanide derivatives, 13a or 13b, or the O-tosylated TmobNH-protected precursor, 22. The substitution of O-Ts with \([^{18}F]\)fluoride using O-tosylated cyanide derivatives, 13a or 13b, was performed with 18-crown-6/KHCO\(_3\) in DMSO at 110 °C for 10 min, followed by a solid-phase extraction (Oasis HLB 3 cm\(^3\) cartridge). The intermediate was eluted from this cartridge and treated with \(H_2SO_4/TFA\) at 120 °C for 10 min. The crude labeled products were cooled to room temperature and neutralized with a saturated \(Na_2CO_3\) solution. The mixture was passed through an Oasis HLB 3 cm\(^3\) cartridge topped with Ag\(_{11}\)-A8 resin. The cartridge was eluted with phosphate buffered saline (pH 7.0) to give the desired radioactive \([^{18}F](2S,4R)-4-FPGln, 3\), and \([^{18}F](2S,4S)-4-FPGln, 4\) (Scheme 3). The purity was measured with reversed-phase HPLC (radiochemical purity) and chiral HPLC (optical purity). (See Figure 3.) The decay-corrected radiochemical yield was 6.2 ± 3.9%, radiochemical purity 91.5 ± 1.5%, optical purity >99%, n = 2 (for 3) and 25.2 ± 2.3%, RCP 92.8 ± 2.6%, optical purity >99%, n = 5 (for 4). It is important to note that radiolabeling of these two seemingly close analogs showed very different yields. We noted the disparity in radiolabeling yields, but we do not have a simple explanation for this phenomenon.
Additional studies may be needed to investigate the optical preferences in the substitution of O-Ts with $[^{18}F]$(fluoride. To improve the radiolabeling reaction for the more promising $[^{18}F]$(2S,4R)-4-FPGln, 4, we made the effort to use a different O-tosylated TmobNH-protected precursor, 22. A similar 4-O-tosylated TmobNH-protected precursor was successfully employed for substitution of O-Ts with $[^{18}F]$(fluoride using similar reaction conditions to give the desired $[^{18}F]$(2S,4R)-4-FPGln, 2, in good radiochemical yields (30–40%). However, much to our surprise, the radiochemical yield for precursor, 22, gave a lower labeling yield. The decay corrected radiochemical yield was 2.7 ± 0.9%, $n = 3$.

**In Vitro Cell Uptake and Inhibition Study in 9L Tumor Cells.** In order to test the specificity of this radiotracer, in vitro cell uptake and inhibition studies were performed in 9L cells. Both $[^{18}F]$(2S,4R)-4-FPGln, 3, and $[^{18}F]$(2S,4S)-4-FPGln, 4, displayed excellent uptake in the 9L tumor cells in vitro. At all time points studied (5 to 120 min), both tracers displayed very similar values (Figure 4). It appeared that the stereoisomers 4S and 4R have comparable tumor cell uptakes. Because of this observation, we only used the $[^{18}F]$(2S,4S)-4-FPGln, 3, and $[^{18}F]$(2S,4R)-4-FPGln, 4, for further investigations on inhibition of cell uptakes and for the in vivo biological studies. The tracer, $[^{18}F]$(2S,4S)-4-FPGln, 4, was incubated at 37 $^\circ$C for 30 min with different amino acid transport inhibitors. The results in Figure 5 suggested that the system A inhibitor, MeAIB (N-methyl-$\alpha$-aminoisobutyric acid), had no inhibitory effect on the uptake, indicating that the system A amino acid transport was not involved in the uptake of this new tracer. System L inhibitor, BCH (2-amino-bicyclo[2.2.1]heptane-2-carboxylic acid), System ASC inhibitor L-serine (L-Ser) and System ASC (SLC1A5), N inhibitor, L-glutamine (L-Gln), exhibited similar concentration dependent reduction of cell uptake, thus indicating potential involvement of system L, ASC, and N in the uptake (Figure 5).

**Protein Incorporation of $[^{18}F]$(2S,4S)-4-FPGln, 4, into 9L Tumor Cells.** One of the important issues to consider when developing tracers to image glutamine metabolism in tumors is the protein incorporation of the tracer once inside the cells. After incubation of $[^{18}F]$(2S,4S)-4-FPGln, 4, and $[^{18}F]$(2S,4R)-4-FPGln with 9L tumor cells, the cell lysates were treated with TCA and the radioactivity in the precipitates and supernatant were counted. Results showed that the majority of $[^{18}F]$(2S,4S)-4-FPGln activity was associated with the TCA precipitates suggesting that most of the $[^{18}F]$(2S,4S)-4-FPGln (>90%) was incorporated into macromolecules, whereas the glutamine analog, $[^{18}F]$(2S,4R)-4-FPGln, 4, remained predominantly in the supernatant (no incorporation).

On the basis of the protein incorporation data above (Figure 6), $[^{18}F]$(2S,4S)-4-FPGln, 4, behaved very differently from that of $[^{3}H]$(Gln. It is reasonable to conclude that the new probe, $[^{18}F]$(2S,4S)-4-FPGln, 4, is not associated with intracellular macromolecules, and thus, it is less likely to measure the intracellular metabolism associated with glutamine metabolism.

**Biodistribution in F344 Rats Bearing 9L Tumor.** Biodistribution studies of $[^{18}F]$(2S,4S)-4-FPGln, 4, were conducted in F344 rats (125–149 g, $n = 4$) bearing 9L tumors on their thigh. This is a well-established animal model that resembles typical human glioblastomas in clinical settings. Rats were sacrificed at 30 and 60 min postinjection by cardiac excision while under isoflurane anesthesia. $[^{18}F]$(2S,4S)-4-FPGln, 4, showed respectable uptake within the 9L tumors, displaying 0.83% dose/g uptake at 30 min post injection. Tumor uptake and retention slowly washed out of the 9L tumor to 0.60% dose/g. At 30 min, tumor-to-background (tumor-to-muscle, tumor-to-blood, and tumor-to-brain) ratios of $[^{18}F]$(2S,4S)-4-FPGln, 4, were 6.91, 1.45, and 5.53, respectively. The highest uptake of $[^{18}F]$(2S,4S)-4-FPGln, 4, was found in the pancreas. High pancreatic uptake is consistent with the fact that amino acids are precursors for digestive enzymes actively produced in the pancreas. Low bone (femur) uptake was observed at 30 min (0.53% dose/g) and it stayed at that value at 60 min post injection.

Preliminary PET imaging studies of $[^{18}F]$(2S,4S)-4-FPGln, 4, in rats with 9L tumors showed that the probe was clearly taken up by the tumors ($n = 3$) (Figure 7). To further investigate the tumor uptake, dynamic small animal PET studies using one rat bearing two 9L tumors were carried out on two different days with either $[^{18}F]$(2S,4S)-4-FPGln, 4, or $[^{18}F]$(2S,4R)-4-FPGln, 2. The direct comparison study using the same animal can avoid some of the complications related to differences in tumor growth in different animals. $[^{18}F]$(2S,4R)-4-FPGln, 2, was recently reported as a tumor PET imaging agent for glutaminolysis. PET images of $[^{18}F]$(2S,4S)-4-FPGln, 4, and $[^{18}F]$(2S,4R)-4-FPGln, 2, were selected for visualization (Figure 7). As these images demonstrate, the 9L tumors could be visualized with either of the ligands. High kidney, liver, and bladder uptake were also observed. Defluorination/bone uptake was more apparent in the images of $[^{18}F]$(2S,4R)-4-FPGln, 2, compared to those of $[^{18}F]$(2S,4S)-4-FPGln, 4. To assess the in vivo kinetics, region-of-interest analysis was performed (using AMIDE software to generate the time-activity curves; Figures 8 and 9). The kinetic curves confirmed that all the tracers exhibited higher tumor uptake compared to the muscle (background) regions. $[^{18}F]$(2S,4S)-4-FPGln, 4, showed a higher tumor-to-muscle ratio than $[^{18}F]$(4-FGln. Both ligands displayed similar kinetics. Both ligands had rapid tumor uptake and reached their maximum uptake within the first 20 min. Tumor uptake for $[^{18}F]$(4-FGln remained rather consistent over 2 h, whereas $[^{18}F]$(2S,4S)-4-FPGln, 4, displayed a faster tumor washout rate. Also noteworthy, $[^{18}F]$(2S,4S)-4-FPGln, 4, showed less defluorination/bone uptake in comparison to that of $[^{18}F]$(4-FGln. Results of the in vivo PET imaging studies using the 9L tumor model suggested that the $[^{18}F]$(2S,4S)-4-FPGln, 4, localized in the 9L tumor as well, if not better, than $[^{18}F]$(2S,4R)-4-FPGln, 2.

![Figure 4](image-url)
DISCUSSION

Glutamine is found circulating in the blood as well as stored in skeletal muscles in high concentrations (0.5–1 mmol/L). Glutamine plays various critical functions: as an energy source and a substrate for DNA and protein synthesis, a primary source of fuel for cells lining the inside of the small intestine and rapidly dividing immune cells, and as a regulator of acid–base balance by producing ammonium in the kidneys. In the brain, the glutamine–glutamate shunt is a critical pathway to control the inhibitory and excitatory neuronal signals. Glutamine transporters play an important role in regulating mammalian cell functions. There are three known glutamine transporters, SLC1A5 (ASCT2, Km 20 mM), LAT1 (SLC7A5, Na+ independent), and SNAT (Na+/neutral transporter).16−19 In the context of tumor growth, SLC1A5 is the most important glutamine transporter responsible for rapidly growing tumors. In these tumor cells, the expression of SLC1A5 is up-regulated.

Table 1. Tissue Distribution of Radioactivitya in F344 Rats Bearing 9L Tumors after Intravenous Injection of [18F](2S,4S)-4-FPGln, 4b

| organ           | 30 min  | 60 min  |
|-----------------|---------|---------|
| blood           | 0.57 ± 0.02 | 0.38 ± 0.02 |
| heart           | 0.31 ± 0.01 | 0.26 ± 0.02 |
| muscle          | 0.12 ± 0.01 | 0.11 ± 0.01 |
| lung            | 0.52 ± 0.01 | 0.39 ± 0.02 |
| kidney          | 12.4 ± 1.02 | 8.93 ± 0.42 |
| pancreas        | 3.22 ± 0.42 | 2.24 ± 0.06 |
| spleen          | 0.59 ± 0.02 | 0.42 ± 0.03 |
| liver           | 1.72 ± 0.07 | 1.58 ± 0.08 |
| skin            | 0.47 ± 0.15 | 0.37 ± 0.06 |
| brain           | 0.15 ± 0.00 | 0.16 ± 0.01 |
| bone            | 0.53 ± 0.08 | 0.56 ± 0.17 |
| 9L tumor        | 0.83 ± 0.04 | 0.60 ± 0.06 |
| tumor/blood     | 1.45 ± 0.08 | 1.57 ± 0.17 |
| tumor/muscle    | 6.91 ± 0.66 | 5.45 ± 0.73 |

aPercent dose/gram. bResults are expressed as mean ± SD (n = 4).
Just as FDG-PET is useful for imaging tumors in which the glucose transporter is overexpressed, glutamine tracers will accumulate in these tumors. The tracer reported in this project appeared to be more sensitive to the inhibition by LAT inhibitor, BCH, not glutamine (SLC1A5). The relationship between amino acid transporter expression and tumor growth is a rapidly expanding research field. Many amino acid derivatives have been reported for imaging tumor growth based on different amino acid transporters,

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FSPG is a tracer useful for assessing system xC. A structural analog of glutamine containing a 3-fluoropropyl substitution group at the C4 position. The mechanisms of uptake and retention are dramatically different.

Further evaluate the significance of the observation on different levels of inhibition by LAT vs SLC1A5 subtypes of amino acid transporters. [18F](2S,4R)-4-fluoroglutamic acid, BAY 85–8050 has been reported as a tumor imaging agent. In order to improve the in vivo stability and to reduce defluorination in vivo, (4S)-4-(3-fluoropropyl)-l-glutamate (18F-FSPG, or BAY 94–9392) was also prepared and tested. It was found that 18F-FSPG, a glutamic acid containing a 3-fluoro-propyl substitution group at the C4 position, showed good tumor uptake and reduced in vivo defluorination. In vivo human studies suggest that 18F-FSPG is a tracer useful for assessing system xC (anionic amino acid) transporter activity in tumors with PET. Piramal Biotechnology is now developing the 18F-FSPG for imaging tumors in which xC transporters are prominently expressed and oxidative stress is up-regulated.

The new (2S,4S)-4-FPGln, 4, reported in this paper, is a structural analog of glutamine containing a 3-fluoro-propyl substitution group at the C4 position. The mechanisms of uptake for [18F](2S,4S)-4-FPGln, 4, are associated with three main amino acid transporters, SLC1A5 (ASCT2), LAT1 (SLC7A5, Na+ independent), and SNAT (Na+/neutral transporter). Based on the inhibition studies, it appears that LAT inhibition was the most prominent, suggesting that LAT may be a preferred transporter for (2S,4S)-4-FPGln, 4. For [13C]Gln, 1, and (2S,4R)-4-FGln, 2, the most important amino acid transporter appeared to be SLC1A5 (ASCT2).

In vitro incubation of [18F](2S,4S)-4-FPGln, 4, with 9L tumor cells showed a high cell uptake reaching 6% uptake/100 µg of protein at 120 min after incubation. Under the same incubation conditions, the lysate of the 9L cells showed that a significant portion of the [18F](2S,4S)-4-FPGln, 4, inside the cells remained intact as the original chemical species (>90% showed no metabolic changes). The [3H]-Gln incubated simultaneously under the same conditions showed substantial incorporation into macromolecules (>90% activity associated with the TCA precipitated fraction). Previously, using the same procedure [13C]Gln, 1, and [18F](2S,4R)-4-FGln, 2, also displayed very similar incorporations into the macromolecular fraction as that observed for [3H]-Gln.7,8 The results suggest that [18F](2S,4S)-4-FPGln, 4, may be more similar to the neutral LAT preferred amino acid analogs, such as O-2-[18F]fluoroethyl)-l-tyrosine (FET).53,54 The uptake mechanism may be overlapping that of [18F]FACBC.25,26 All of these amino acid probes are transported into the cancer cells, without being incorporated into intracellular macromolecules. There are important differences between these seemingly very close glutamine analogs, [18F](2S,4R)-4-FGln, 2, and [18F](2S,4S)-4-FPGln, 4. Further exploration may be needed to clarify the similarities and differences between these probes. These development of effective probes for studying glutamine metabolism, one should consider more than simple factors, such as tumor cell uptake and in vivo tumor signal localization. It may also be necessary to consider the subsequent intracellular metabolic processes, or the lack thereof. Compared to the "natural" [13C]Gln, 1, fluorine substituted [18F](2S,4R)-4-FGln, 2, or [18F](2S,4S)-4-FPGln, 4, may always be suspected of having a modified intracellular metabolism. More studies are necessary to characterize these analogs for studying glutamine metabolism in tumors.

The most important difference between [18F](2S,4R)-4-fluoroglutamic acid (BAY 85–8050) and [18F](2S,4S)-4-(3-fluoro-propyl)glutamic acid (18F-FSPG, or BAY 94–9392) is that 18F-FSPG, displays a slower defluorination rate in vivo. Preliminary human studies have demonstrated that the bone marrow uptake in the vertebral region is relatively low.31,32 The same scenario may or may not apply to the second pair of glutamine probes (Figure 10). We have noticed a reduced bone uptake (of 4) in rats as compared to the uptake of [18F](2S,4S)-4-FPGln, 4, as visualized by PET or by a dissection method. Results from both methods suggest a reduced bone uptake (of 4) in rats as compared to the uptake of [18F](2S,4R)-4-FGln, 2.8 Loss of fluoride is a constant concern for fluoro-alkyl labeled radiopharmaceuticals. Our observation suggests that defluorination is not an issue for 4.

In summary, a new glutamine analog, [18F](2S,4S)-4-FPGln, 4, has shown tumor specific uptake in vitro and in vivo. However, the tumor uptake and retention mechanisms may be significantly different from other glutamine probes, such as [13C]Gln, 1, and [18F](2S,4R)-4-FGln, 2.

### AUTHOR INFORMATION

**Corresponding Author**

*Email: kunghf@sunmac.spect.upenn.edu. Phone: 215-662-3096. Fax: 215-349-5035.*

**Author Contributions**

*Authors Z.W. and Z.Z. contributed equally to this paper.*
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