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# (*R*,*S*)-*anti*-1-Amino-2-[$^{18}$F]Fluorocyclopentyl-1-carboxylic Acid: Synthesis from Racemic 2-Benzyloxycyclopentanone and Biological Evaluation for Brain Tumor Imaging with Positron Emission Tomography

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(*R*,*S*)-*anti*-1-Amino-2-fluorocyclopentyl-1-carboxylic acid (2-FACPC, **4b**) was radiolabeled in 39% yield starting from cyclic sulfamidate **12**. The 9L gliosarcoma cells assays showed that **4b** is mainly a substrate for the L-type amino acid transport with some affinity to the A-type. In rats bearing 9L gliosarcoma tumors, **4b** displayed high tumor to brain ratio (10:1) at 120 min after injection. FACPC is an attractive candidate for imaging brain tumors with PET, and its isolated enantiomers are under investigation.

## Introduction

Positron emission tomography (PET) is an excellent technique for examining various biochemical processes in malignant tissues in vivo by applying certain radiotracers.[1] 2-[$^{18}$F]Fluoro-2-deoxy-D-glucose ([$^{18}$F]FDG) is currently the most widely used radiotracer in tumor diagnosis using PET-CT in clinic.[1a] Despite the success of [$^{18}$F]FDG-PET in clinical oncology, [$^{18}$F]FDG-PET imaging has several important limitations. The high physiological uptake of [$^{18}$F]FDG in normal tissues such as the brain and urinary tract can complicate the diagnostic evaluation of tumors in these locations. Numerous infectious and noninfectious inflammatory conditions lead to increased local [$^{18}$F]FDG uptake which can result in false positive studies.[2] In contrast, the natural carbon-11 ($^{11}$C) amino acid L-[methyl-$^{11}$C]methionine ([$^{11}$C]MET)[3] has been shown to display good tumor uptake accompanied by lower uptake in inflammatory cells than [$^{18}$F]FDG. This indicated that amino acid tracers could potentially better differentiate tumor from inflammation.[4] In an effort to overcome the disadvantages of [$^{18}$F]FDG, many natural and non-natural amino acid analogues labeled with $^{11}$C[5] or fluorine-18 ($^{18}$F)[5,6] have been developed including the $^{18}$F labeled non-natural amino acid *anti*-1-amino-3-[$^{18}$F]fluorocyclobutane-1-carboxylic acid ([$^{18}$F]-FACBC) prepared in our laboratory.[6,7] However, there have been some limitations such as the following: the $^{11}$C short half-life (20 min) limits the use of the $^{11}$C analogues to medical centers having an on-site cyclotron, and several of the $^{18}$F derivatives[8] suffer from low radiochemical and/or synthetic yields. 1-Aminocyclopentane-1-[$^{11}$C]carboxylic acid ([$^{11}$C]ACPC) has been reported for imaging brain tumors.[9] ACPC has similar transport characteristics compared to MET: it is transported across cell membranes by the L-type (leucine preferring, sodium-independent) transport system and has some affinity to the A-type (alanine preferring, sodium-dependent). In contrast to MET, ACPC as a non-natural amino acid is not metabolized or incorporated into proteins in mammals. Therefore, the problems associated with imaging radiolabeled metabolites are avoided.[9a] To address those shortcomings, we sought to develop (*R*,*S*)-*anti*-1-amino-2-[$^{18}$F]fluorocyclopentyl-1-carboxylic acid (2-[$^{18}$F]FACPC) as a PET fluorinated analogue of ACPC for detecting brain tumors. Herein, we report the synthesis, $^{18}$F labeling, and biological characterization of the racemic mixture of FACPC to evaluate its feasibility as a new brain tumor imaging agent.

## Results and Discussion

**Chemistry.** Compounds **1**−**12** were prepared as shown in Schemes 1 and 2 and in ways similar to the methods previously reported.[10] The key step in the preparation of the fluorinated amino acid **4b** involved the synthesis of the benxyloxy hydantoin intermediates **2a** and **2b** using a modified Bucherer−Bergs−Strecker reaction.[11] Treatment of **1**[10a] with ammonium carbonate, ammonium chloride, and potassium cyanide provided a mixture of **2a** (*syn*) and **2b** (*anti*) in approximately an 8:1 *syn*/*anti* ratio. It is interesting to note that based on the literature,[11] the formation of the benzyloxy derivative is stereoselective and the major isomer possesses the *syn* conformation. Following chromatographic purification, hydantoin **2b** was converted to the *anti*-amino acid **4b** as depicted in Scheme 1. No attempt was made to synthesize the *syn* isomer.

The cyclic sulfamidate labeling precursor **12** was synthesized from the freshly prepared siloxycyclopentene **5**[10f] as shown in Scheme 2. Under the appropriate conditions, **6** provided *syn*-**7** as the major product.[11] Alkaline hydrolysis of **7** followed by treatment with di-*tert*-butyl dicarbonate and *tert*-butyl-2,2,2-trichloracetimidate afforded **9** which, after three steps including a catalytic debenzylation, provided the cyclic sulfamidate **12**, which is the radiolabeling precursor for [$^{18}$F]FACPC, in satisfactory yield. In our case, the 2-position of ACPC is not readily susceptible to $S_N2$ halogen substitution because of its neopentyl character. The use of the cyclic sulfamidate precursors for the $^{18}$F labeling, in such cases, was discussed in previous work.[10d,e]

**Radiochemistry.** The radiofluorinated target *anti*-[$^{18}$F]-FACPC ([$^{18}$F]**4b**) was prepared, as previously reported,[10e]

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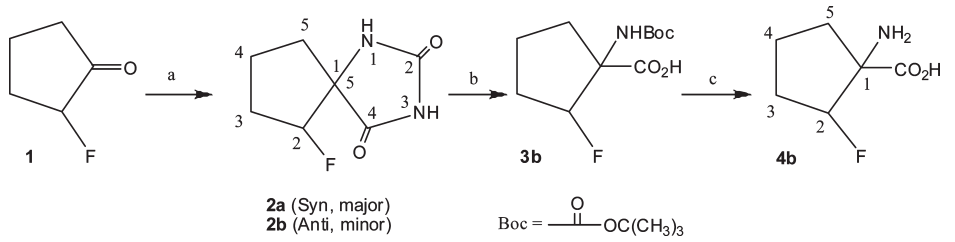
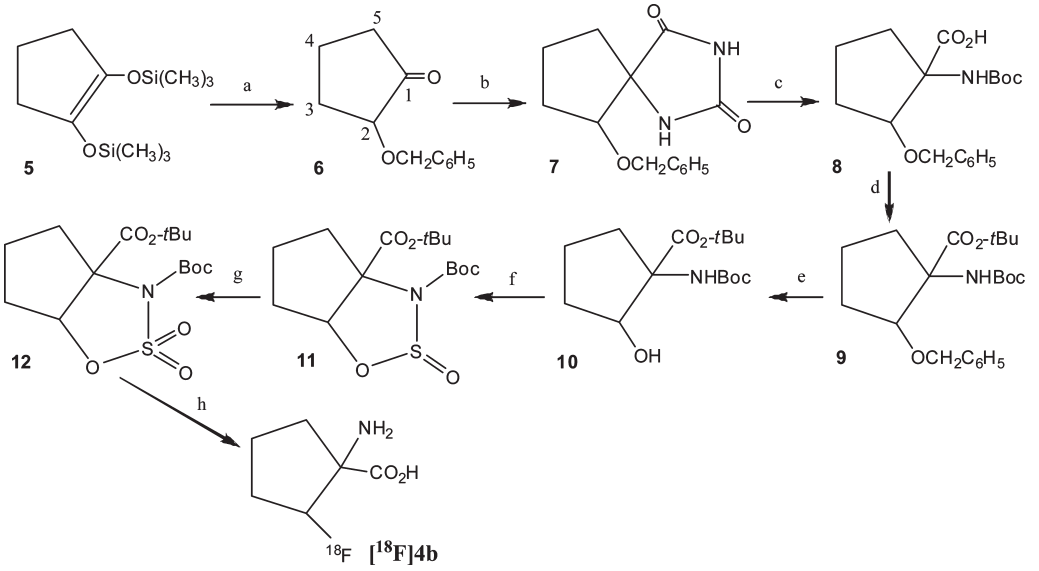
**Scheme 1.** Synthesis of Flurorine-19 Amino Acid FACPC (**4b**)[a]




[a] Reagents: (a) $(NH_4)_2CO_3$, $NH_4Cl$, KCN, 1:1 $EtOH/H_2O$; (b) 3 N NaOH, 120 °C, and then $Boc_2O$, 9:1 $CH_3OH/Et_3N$; (c) 4 N HCl, 100 °C. **1** was prepared according to a published procedure: *Org. Lett.* **1999**, *1* (10), 1591−1594.

**Scheme 2.** Synthesis of Cyclic Sulfamidate (**12**) and Radiosynthesis of [$^{18}$F]FACPC ([$^{18}$F]**4b**)[a]




[a] Reagents and conditions: (a) BnOH, HCl/ether; (b) $(NH_4)_2CO_3$, $NH_4Cl$, KCN, 1:1 $EtOH/H_2O$; (c) 3 N NaOH, 120 °C, and then $Boc_2O$, 9:1 $CH_3OH/Et_3N$; (d) $Cl_3CC(=NH)OtBu$, $CH_2Cl_2$; (e) 10% Pd/C, $H_2$, $CH_3OH$; (f) $SOCl_2$, Py, $CH_3CN$; (g) $NaIO_4$, catalytic $RuO_2.H_2O$, $CH_3CN$; (h) [$^{18}$F]HF, $K_2CO_3$, $K_{222}$, $CH_3CN$, 110 °C and then 6 N HCl, 110 °C.

starting from precursor **12** (1 mg) by no-carrier-added nucleophilic substitution using dried K[$^{18}$F]F, potassium carbonate, and Kryptofix in acetonitrile followed by deprotection with 6 N HCl (Scheme 2). The crude $^{18}$F labeled product was purified by passing through a series of an ion-retardation resin column followed by an alumina N SepPak, to remove the [$^{18}$F]fluoride, radiolabeled ionic byproducts, and the hydrogen chloride from the hydrolysis step, and an HLB Oasis reverse-phase cartridge to remove the less polar and organic byproducts. The purified [$^{18}$F]**4b** was obtained in a pH 6−7 normal saline solution and used directly in the in vitro and in vivo studies. The average radiochemical decay-corrected yield (dcy) was 39 ± 8% ($n$ = 4). Radiometric TLC of [$^{18}$F]**4b** showed a single radiolabeled product in a radiochemical purity >99% and an $R_f$ corresponding to the authentic fluorine-19 compound. The total synthesis time was 60 min from end of bombardment. Since the specific activity of [$^{18}$F]**4b** was not directly determined, the maximum amount of nonradioactive material in the final dose arising from the precursor is about 1 mg. On the basis of a 217 mCi yield at end of synthesis, the amount of unlabeled material in the final product arising from the cyclic sulfamidate precursor would not exceed 5 μg/mCi. This amount of unlabeled material is comparable to the amount present in doses of other $^{18}$F amino acid tracers.[10c,d]

**Amino Acid Transport Assays.** The biological transport properties of [$^{18}$F]**4b** were examined through uptake assays using cultured rat 9L gliosarcoma tumor cells in the absence and presence of amino acid transport inhibitors. The non-natural amino acid 2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH, L-type transport inhibitor) and *N*-methyl-α-aminoisobutyric acid (MeAIB, A-type transport inhibitor) were used. A mixture of alanine, cysteine, and serine (ACS) was also used to provide a wide spectrum of amino acid transport inhibition to define the entry of [$^{18}$F]**4b** into the 9L cells by pathways other than amino acid transport. The uptake data were normalized and expressed as mean percent ligand uptake relative to the no inhibitor (control) condition (Table 1). In the absence of inhibitors, [$^{18}$F]**4b** showed intracellular accumulation of 6.33 ± 0.47% ($n$ = 3) of the initial dose per half a million cells after 30 min of incubation in 9L gliosarcoma cells. In the presence of the ACS mixture, 65% of the cellular uptake of [$^{18}$F]**4b** was blocked relative to no inhibitor ($p$ < 0.01). This result indicated that the vast majority of entry of [$^{18}$F]**4b** into the 9L tumor cells in vitro occurred via amino acid transport system rather than a passive process. The uptake of [$^{18}$F]**4b** was reduced by 38% relative to no inhibitor in the presence of MeAIB ($p$ < 0.01), while in the presence of BCH the inhibition was significantly greater with 71% inhibition relative to control ($p$ < 0.001). These inhibition studies suggested that [$^{18}$F]**4b**, like its $^{11}$C parent the [$^{11}$C]ACPC, is predominantly an L-type substrate with some affinity to the A-type. Furthermore, because [$^{18}$F]**4b** was evaluated as a

**Table 1.** 9L Gliosarcoma Cell Uptake of [$^{18}$F]**4b** with or without Inhibitors after 30 min of Incubation[a]

| | control | BCH | MeAIB | ACS |
|---|---|---|---|---|
| uptake (%) | 6.33 ± 0.47 | 1.84 ± 0.20 | 3.94 ± 0.19 | 2.25 ± 0.27 |
| inhibition (%) | | 71 | 38 | 65 |
| *P* | | < 0.001 | < 0.01 | < 0.01 |

[a] Data are presented as percent ligand uptake of the initial dose per 0.5 million cells (% uptake/(0.5 × $10^6$ cells)) ± standard deviation (SD) ($n$ = 3) and normalized for the dose and number of cells. Inhibition is expressed as percent relative to control. $p$ values represent comparisons of uptake in the presence of inhibitor to control uptake using one-tailed paired $t$-test.

racemic mixture, it is possible that its isolated enantiomers differ in their specificity for the various amino acid transport systems. The separation and a more detailed analysis of the biological transport properties of those enantiomers in a panel of different human tumor cell lines are underway.

**Biodistribution Studies in Rats with Intracranial 9L Gliosarcoma Tumors.** The tissue distribution of [$^{18}$F]**4b** after tail injection in Fisher rats bearing 9L gliosarcoma implants is shown in Table 2. Tumor uptake of radioactivity, after injection of [$^{18}$F]**4b**, at 15, 30, 60, and 120 min was 0.57, 1.68, 1.24, and 0.98% injected dose per gram tissue (%ID/g), respectively, and was significantly higher than in the normal brain contralateral tissue which was approximately 0.11 % ID/g at all time points ($p < 0.01$ using two-tailed paired $t$-test). It is interesting to note that the brain displayed the lowest uptake of radioactivity and stayed stable (around 0.11 %ID/g) through the course of the 120 min study. The resulting ratios of tumor to normal brain uptake were 5:1, 13:1, 12:1, and 10:1 at 15, 30, 60, and 120 min, respectively. The high tumor uptake of [$^{18}$F]**4b** in vivo was consistent with the results obtained with [$^{14}$C]ACPC in rat glioma experiments where the results showed higher accumulation of radioactive ACPC in the rat glioma tumors than in the contralateral normal brain. Those observations were explained by the fact that the facilitated transport of ACPC is up-regulated across the glioma capillaries and that tumors can induce an "up-regulation" of the amino acid transporter expression in their supporting vasculature.[9a] Similar high tumor to normal brain ratios were obtained with other fluorinated amino acid radiotracers, which are also L-type transport substrates.[10c] As in the in vitro cell studies, those findings showed the high uptake of [$^{18}$F]**4b** by the 9L tumors.

In normal tissues, [$^{18}$F]**4b** displayed the highest uptake of radioactivity in the pancreas (2.65 %ID/g) followed by the kidneys (2.00 %ID/g) at 60 min, similar to other studies with A-type and L-type amino acid imaging agents.[10e,12] Additionally, the rest of the measured tissues (heart, lung, liver, spleen, muscle, and testes) exhibited relatively low uptake, suggesting that [$^{18}$F]**4b** may be suitable to image tumors outside the central nervous system. The lack of significant accumulation of radioactivity in the bone indicated that no in vivo defluorination occurred during the 120 min study.

The absolute values of radioactivity uptake in the tumor and normal brain, achieved 60 min after [$^{18}$F]**4b** injection, are similar to those reported for [$^{18}$F]FACBC in the same animal model and at the same time point.[7b] At 60 min, [$^{18}$F]**4b** and [$^{18}$F]FACBC uptakes in the tumor were 1.24 and 1.72 %ID/g, respectively. At this time, the tumor to brain ratio exhibited by [$^{18}$F]**4b** (12:1) (Table 2) was higher than that reported by [$^{18}$F]FACBC (6.6:1).[7b] When [$^{18}$F]FDG was evaluated in the same animal model, the uptake in the tumor was 1.05 %ID/g at 60 min and the tumor to brain ratio was 0.84:1 at this time.[7b] [$^{18}$F]**4b** and [$^{18}$F]FACBC showed higher uptake in tumor tissue and lower uptake in normal brain than [$^{18}$F]FDG.

**Table 2.** Biodistribution as Percent of Injected Dose per Gram (%ID/g) of Radioactivity in Tissues of 9L Tumor-Bearing Fisher Rats following Intravenous Administration of [$^{18}$F]**4b**[a]

| tissue | 15 min | 30 min | 60 min | 120 min |
|---|---|---|---|---|
| blood | 0.48 ± 0.06 | 0.29 ± 0.04 | 0.21 ± 0.02 | 0.14 ± 0.04 |
| heart | 0.56 ± 0.03 | 0.44 ± 0.08 | 0.30 ± 0.11 | 0.28 ± 0.08 |
| lung | 1.53 ± 0.16 | 1.13 ± 0.41 | 0.80 ± 0.22 | 0.78 ± 0.33 |
| liver | 0.93 ± 0.10 | 0.63 ± 0.07 | 0.41 ± 0.10 | 0.40 ± 0.17 |
| pancreas | 2.77 ± 0.47 | 2.91 ± 0.63 | 2.65 ± 0.65 | 1.84 ± 0.24 |
| spleen | 0.64 ± 0.04 | 0.48 ± 0.15 | 0.30 ± 0.08 | 0.18 ± 0.02 |
| kidney | 4.87 ± 1.19 | 3.44 ± 1.44 | 2.00 ± 0.52 | 0.89 ± 0.11 |
| muscle | 0.28 ± 0.05 | 0.28 ± 0.07 | 0.24 ± 0.03 | 0.17 ± 0.05 |
| brain | 0.12 ± 0.03* | 0.13 ± 0.06** | 0.10 ± 0.03*** | 0.10 ± 0.03‡ |
| tumor | 0.57 ± 0.00* | 1.68 ± 0.45** | 1.24 ± 0.37*** | 0.98 ± 0.33‡ |
| testes | 0.23 ± 0.02 | 0.18 ± 0.03 | 0.14 ± 0.03 | 0.13 ± 0.04 |
| bone | 0.27 ± 0.03 | 0.17 ± 0.02 | 0.14 ± 0.03 | 0.13 ± 0.02 |
| tumor/brain ratio | 5:1 | 13:1 | 12:1 | 10:1 |

[a] Values are reported as mean percent dose per gram ± standard deviation (SD) ($n$ = 5) at each time point. $p$ values were determined using two-tailed paired $t$-test: (*) $p < 0.01$; (**) $p < 0.001$; (***) $p < 0.001$; (‡) $p < 0.01$.

It is important to consider the role of the blood−brain barrier (BBB) when evaluating the biodistribution of novel radiolabeled amino acids for tumor imaging. Charged molecules such as amino acids do not readily cross the normal BBB without biological transport, and high tumor to normal brain ratios could potentially be due to disruption of the BBB in the tumor vasculature.[10c] In the case of [$^{18}$F]**4b**, both the in vitro and in vivo studies demonstrate that the title non-natural amino acid is a substrate for biological transport. To avoid the potential confound of low uptake in normal brain tissue due to an intact BBB, skeletal muscle was used as a normal tissue for comparison. The tumor to muscle ratio was 5.2:1 at 60 min after [$^{18}$F]**4b** injection. This value was similar to that for [$^{18}$F]FDG and higher than that for [$^{18}$F]FACBC which displayed tumor to muscle ratios of 5.25:1 and 4.1:1, respectively, at the same time point and in the same animal model.[7b] Furthermore, uptake of activity after [$^{18}$F]FDG injection was 1.05 %ID/g in tumor tissue at 60 min vs 1.24 % ID/g for [$^{18}$F]**4b** at the same time point (Table 2). If [$^{18}$F]**4b** was entering the tumor tissue through diffusion only, a lower absolute uptake amount would be expected compared to [$^{18}$F]FDG. Altogether, those data demonstrate that the tumor to brain ratios obtained with [$^{18}$F]**4b** are due to biological transport and do not simply reflect heterogeneous permeability of the BBB.

## Conclusions

The racemic mixture of 2-FACPC has been synthesized and radiolabeled with fluorine-18 in good radiochemical yield (39% dcy) and high radiochemical purity from a stable precursor using a semiautomated synthesis system. The inhibition experiments demonstrated that [$^{18}$F]**4b** entered the 9L tumor cells in vitro primarily via the L-type amino acid transport and underwent some A-type transport. The in vivo biodistribution studies showed that [$^{18}$F]**4b** displayed high uptake and prolonged retention in rat 9L gliosarcoma intracranial tumors and low uptake in the normal brain tissue. These results were similar to those obtained with [$^{18}$F]FACBC in the same animal model with[$^{18}$F]**4b** displaying higher tumor to brain ratio than [$^{18}$F]FACBC at 60 min.

The very high tumor to brain ratios and in vivo stability to defluorination suggest that 2-FACPC is an excellent candidate for further evaluation for imaging tumors in humans. Because [$^{18}$F]**4b** was evaluated as a racemic mixture, it is possible that the single enantiomers would exhibit different biodistribution profiles. Studies are underway to determine the transport properties of the isolated enantiomers of [$^{18}$F]**4b** in a variety of human cancer cell lines.

## Experimental Section

**Materials and Instrumentation.** Reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) with purities of >99% and used without further purification. Thin-layer chromatography (TLC) analyses were performed using 250 $\mu$m UV254 silica gel backing on aluminum plates purchased from Whatman Ltd. (Maidstone, Kent, U.K.). Flash column chromatography was carried out using Merck Kieselgel silica gel 60 (230−400 mesh). Proton nuclear magnetic resonance ($^1$H NMR) spectra were run on a Mercury Unit 300 at 300 MHz in $CDCl_3$ with tetramethylsilane (TMS) as internal standard at Emory University CSI. Elemental analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA), and the purities of the tested compounds were >95% unless otherwise indicated. Mass spectra were run on a JEOL JMS-SX102/SX102A/E or VG 70-S double focusing mass spectrometer using high-resolution electrospray ionization (ESI). The animal experiments were carried out in compliance with the Emory Institutional University Animal Care Committee (IUCAC) and Radiation Safety regulations. The [$^{18}$F]fluoride was produced at Emory University CSI with an 11 MeV Siemens RDS 111 negative-ion cyclotron (Knoxville, TN) by the $^{18}$O(p, n)$^{18}$F reaction using [$^{18}$O]$H_2O$ (95%). Alumina N SepPaks and HLB Oasis cartridges were purchased from Waters, Inc. (Milford, MA). The ion retardation (IR) chromatography columns and the IR resin AG 11A8 (50−100 mesh) were purchased from Bio-Rad Laboratories (Hercules, CA). Trap/release cartridges model DW-TRC were purchased from D&W, Inc. (Oakdale, TN). Radiometric TLC was performed with the same type of silica plates from Whatman and analyzed using a Raytest system (model Rita Star, Germany). Compound **1** was prepared as previously reported.[10a] The target compound **4b** was prepared as a racemic mixture in both its fluorine-18 and fluorine-19 forms.

**Chemistry. *anti*-1-Amino-2-fluorocyclopentyl-1-carboxylic Acid (4b).** To a solution of **3b** (17 mg, 0.069 mmol) in methanol (1 mL) was added 4 N HCl (1 mL). The reaction vessel was sealed and heated at 100 °C for 20 min. After cooling, the clear pale yellow solution was loaded on an ion retardation resin (AG 11A8) column in series with an alumina N SepPak and a C18 SepPak and eluted with water. The aqueous solution was washed with 3 × 5 mL of $CH_2Cl_2$ and then concentrated under reduced pressure to dryness to provide the amino acid (**4b**) (6.2 mg, 61%). $^1$H NMR ($D_2O$, $\delta$): 1.78 (m, 4H), 2.19 (m, 2H), 4.26 (s, 1H). HRMS, *m*/*z*, calcd for $C_6H_9FNO_2$ [M − H]$^+$, 146.07; found, 146.06878. Anal. ($C_8H_{18}Cl_2FNO_4$) Calcd: C, 34.06; H, 6.43; N, 4.96. Found: C, 34.40; H, 5.76; N, 4.79. The combustion analysis showed that the target compound (**4b**) is solvated; it contains traces amounts of $CH_3OH$, $H_2O$, and $CH_2Cl_2$. The quantity of solvents was included in the compound formula ($C_8H_{18}Cl_2FNO_4$).

**1,2-Bis(trimethylsiloxy)cyclopentene (5).** In a modification to the method previously reported, sodium (0.63 g, 0.027 g·atom) in toluene (10 mL) was reacted with trimethylchlorosilane (3.16 g, 29.08 mmol) and dimethyl glutarate (1.00 g, 6.24 mmol) under reflux conditions and strong stirring for 5 h. The reaction mixture was filtered through silica and rinsed with DCM/$Et_2O$ (80/20). The solvent was evaporated under reduced pressure and at 40 °C to dryness to yield a pale yellow oil (1.30 g, 85%) used without further purification. $^1$H NMR ($CDCl_3$, $\delta$): 0.18 (s, 18H,), 1.76 (q, 2H), 2.24 (t, 4H). HRMS, *m*/*z*, calcd for $C_{11}H_{23}O_2Si_2$ [M − H]$^+$, 243.13; found, 243.122 68.

**2-Benzyloxycyclopentanone (6).** A mixture of benzyl alcohol (0.33 g, 3.06 mmol) and HCl−diethyl ether (1.0 M solution) (1.04 mL) was cooled to 0 °C, and **5** (0.65 g, 2.66 mmol) was added dropwise with stirring. After completion of the addition, the mixture was refluxed for 4 h and concentrated under reduced pressure. Purification by silica gel flash column chromatography using DCM/EtOAc (80/20) afforded **6** as a light yellow oil (0.45 g, 88%). $^1$H NMR ($CDCl_3$, $\delta$): 1.79 (m, 2H), 2.04 (m, 1H), 2.25 (m, 2H), 2.43 (m, 1H), 3.80 (t, 1H), 4.69 (d, 1H, $J$ = 12.0 Hz), 4.83 (d, 1H, $J$ = 12.0 Hz), 7.35 (m, 5H). HRMS, *m*/*z*, calcd for $C_{12}H_{13}O_2$ [M − H]$^+$, 190.10; found, 189.09081.

***syn*-3-(*N*-(*tert*-Butoxycarbonyl)amino)-4-cyclopentane-1,2,3-oxathiazolidine-4-carboxylic Acid *tert*-Butyl Ester 2,2-Dioxide (12).** A solution of **11** (58 mg, 0.17 mmol) in acetonitrile (7 mL) was cooled in an ice bath and treated successively with sodium periodate ($NaIO_4$) (41 mg, 0.19 mmol), a catalytic amount of ruthenium(IV) oxide hydrate ($RuO_2 \cdot H_2O$) (0.4 mg), and $H_2O$ (4 mL). After 30 min of stirring, the ice bath was removed and the reaction was continued for 30 min at room temperature. The reaction mixture was partitioned between EtOAc and water. The aqueous layer was further extracted with EtOAc. Organic layers were combined, washed with saturated $NaHCO_3$ solution and water, dried over $Na_2SO_4$, filtered, and concentrated under reduced pressure. Purification by silica gel flash column chromatography using hexane/EtOAc (90/10) afforded **12** as a clear oil (54.10 mg, 87%). $^1$H NMR ($CDCl_3$, $\delta$): 1.49 (s, 9H), 1.56 (s, 9H), 1.98 (m, 3H), 2.20 (m, 2H), 2.58 (m, 1H), 4.88 (s, 1H). HRMS, *m*/*z*, calcd for $C_{15}H_{29}N_2O_7S$ [M + $NH_4$]$^+$, 381.14; found, 381.16917. Anal. ($C_{15}H_{25}NO_7S$) C, H, N.

**Radiolabeling. *anti*-1-Amino-2-[$^{18}$F]fluorocyclopentyl-1-carboxylic Acid ([$^{18}$F]4b).** The preparation of [$^{18}$F]**4b** was based on the previously reported automated synthesis of *anti*-[$^{18}$F]FACBC.[6] To a glass vessel containing a solution of $K_{222}/CH_3CN$ (2.0 mg/mL) (1.0 mL) was added 1700 mCi ($n$ = 4) of no-carrier-added [$^{18}$F]HF (50 $\mu$A, 60 min bombardment) through a trap/release (T/R) cartridge by using a solution of $K_2CO_3/H_2O$ (1.5 mg/mL) (0.6 mL). The solvent was removed at 110 °C with a nitrogen flow, and an additional $CH_3CN$ (4.0 mL) was added followed by evaporation of the solvent with a nitrogen flow to remove residual $H_2O$. Cyclic sulfamidate precursor **12** (1.0 mg) in dry $CH_3CN$ (0.5 mL) was added to the vial, and the reaction mixture was heated at 110 °C for 10 min. The intermediate product was treated with 6 N HCl (0.5 mL) at 110 °C for 10 min and purified by passing through an IR column assembly consisting of a 7 mm × 120 mm bed of AG 11A8 IR resin column, a neutral alumina SepPak (preconditioned with water (20.0 mL)), and an HLB Oasis reverse phase cartridge (preconditioned with ethanol (10.0 mL) and water (10.0 mL)). [$^{18}$F]**4b**, eluted in series through the assembly with three successive portions of sterile saline (~3.0 mL), passed through a 0.22 $\mu$m sterile filter into a dose vial and was ready for the in vitro and in vivo studies. Further evidence of the identity of [$^{18}$F]**4b** was achieved by comparing the $R_f$ of the radioactive product visualized with radiometric TLC with the $R_f$ of the authentic $^{19}$F compound visualized with ninhydrin stain, using the solvent $CH_3CN/H_2O/CH_3OH$ = 10:5:5 ($R_f$ = 0.64 ± 0.01, Whatman silica gel plates). The only peak present on radiometric TLC analysis corresponded to **4b**, and the radiochemical purity of the product exceeded 99%. The isolated radiochemical yields were determined using a dose calibrator.

**In Vitro Amino Acid Uptake and Inhibition Assays.** These assays were performed with cultured rat 9L gliosarcoma cells as described previously.[10e] Briefly, approximately $0.5 \times 10^6$ cells were exposed to 100 $\mu$Ci of [$^{18}$F]**4b** in amino acid free Hanks' balanced salt solution (HBSS) (0.1 mL) with or without transport inhibitors (10 mM final concentration of BCH, MeAIB, or ACS) for 30 min under incubator conditions in 1.5 mL conical tubes. Each assay condition was performed in triplicate. The data from these studies were presented as percent ligand uptake of the initial dose per $0.5 \times 10^6$ cells and analyzed using one-tailed paired $t$-test.

**Rodent Biodistribution in Tumor Bearing Rats.** Cultured 9L gliosarcoma cells were implanted into the brains of male Fisher 344 rats (200−250 g) for biodistribution studies as described previously.[10e] Briefly, a total of 20 animals were used in the study (5 at each time point). Approximately 10−12 days after implantation, the rats were injected with 40−50 $\mu$Ci of radiotracer [$^{18}$F]**4b** in 0.2−0.4 mL of sterile normal saline via catheters into tail veins and sacrificed at 15, 30, 60, and 120 min after injection. The data were analyzed using two-tailed paired *t*-test.

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**Supporting Information Available:** Experimental abbreviations list; synthesis procedures and data for compounds **2**, **3**, and **7**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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