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Synthesis and biological evaluation of *anti*-1-amino-2-[^{18}F]fluoro-cyclobutyl-1-carboxylic acid (*anti*-2-[^{18}F]FACBC) in rat 9L gliosarcoma

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ABSTRACT

A new [^{18}F] labeled amino acid *anti*-1-amino-2-[^{18}F]fluoro-cyclobutyl-1-carboxylic acid **9** (*anti*-2-[^{18}F]FACBC) was synthesized in 30% decay-corrected yield with high radiochemical purity over 99%. The cyclic sulfamidate precursor was very stable and highly reactive towards nucleophilic radiofluorination. Cell uptake assays with rat 9L gliosarcoma cells showed that [^{18}F]**9** was transported into tumor cells via multiple amino acid transport systems, including L and A systems. Biodistribution study in rats with intracranial 9L gliosarcoma tumors demonstrated that [^{18}F]**9** had a rapid and prolonged accumulation in tumors with 26:1 tumor to brain ratio at 120 min post-injection. In this model, [^{18}F]**9** is a potential PET tracer for brain tumor imaging.

Radiolabeled amino acids, representing a class of metabolically based radiotracers, have been of interest for scientists to study potential imaging characteristics with positron emission tomography (PET) and single photon emission tomography (SPECT).[1–16] Amino acids are important biological substrates in virtually all biological process and are required nutrients for proliferating tumor cells.[6,10,17] Many tumor cells demonstrate increased amino acid transport relative to normal tissues.[18,19]

Amino acids used for tumor imaging can be divided into two major categories, naturally occurring amino acids and their structurally similar analogues, and non-natural amino acids. Non-natural amino acids generally have greater metabolic stability and can be labeled with longer-lived radionuclides such as fluorine-18 for PET imaging. Amino acids enter normal and neoplastic cells primarily through specific membrane associated carrier proteins with varying selectivity based on their chemical structure. For radiolabeled amino acids, tumor uptake is primarily determined by amino acid transport rather than protein synthesis.[6,10] The most abundant amino acid transport systems in most mammalian cells are system A, system L, system ASC, and system Gly.[10,20–25] All of these transport systems are sodium-dependent except system L. Amino acid transport is upregulated in many tumor types. Certain amino acid transporters also are involved in cell signaling and may play important roles in tumor biology.[22,26,27] Additionally, the amino acids developed for tumor imaging may complement the glucose analogue 2-[^{18}F]fluoro-2-deoxyglucose ([^{18}F]FDG), which is the only PET agent currently used for clinical oncology, as FDG has some limitations including high uptake in inflammatory tissue and normal gray matter of the brain. In contrast, non-natural amino acids such as *anti*-1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid (*anti*-[^{18}F]FACBC), has demonstrated lower uptake in the inflamed popliteal lymph nodes and benign prostatic hyperplasia than those for [^{18}F]FDG in a rat prostate cancer model.[28]

A number of [^{18}F] labeled non-natural cyclobutyl amino acids have been prepared in our lab, including *anti*- and *syn*-1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid (*anti*-[^{18}F]FACBC[29,30] and *syn*-[^{18}F]FACBC[31]) and *syn*- and *anti*-1-amino-3-[^{18}F]fluoromethyl-cyclobutane-1-carboxylic acid (*syn*- and *anti*-[^{18}F]FMACBC).[32] These compounds showed high tumor uptake in 9L rat gliosarcoma tumors. Among these amino acids, the PET tracer *anti*-[^{18}F]FACBC is undergoing initial human clinical trials to validate it as a valuable imaging agent for the diagnosis and management of treatment of cancer.[33–35] In an effort to develop more radiotracers for PET tumor imaging, we report herein the synthesis, radiolabeling and biological evaluation of *anti*-1-amino-2-[^{18}F]fluorocyclobutane-1-carboxylic acid (*anti*-2-[^{18}F]FACBC, **9**) with a rat 9L gliosarcoma cell line and in Fischer rats with intracranial 9L tumors. *anti*-2-[^{18}F]FACBC is a constitutional isomer of *anti*-[^{18}F]FACBC, also known as *anti*-3-[^{18}F]FACBC (Fig. 1). On the basis of the observation and the promising results obtained with *anti*- and *syn*-[^{18}F]FACBC and their analogues *anti*- and *syn*-[^{18}F]FMACBC, our goal was to expend the synthetic scope to study the effect of structure of cyclobutyl amino acids on the biological properties of these compounds.

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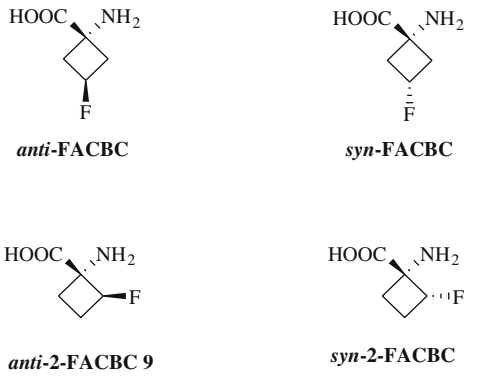


Figure 1. Structures of FACBC and 2-FACBC.

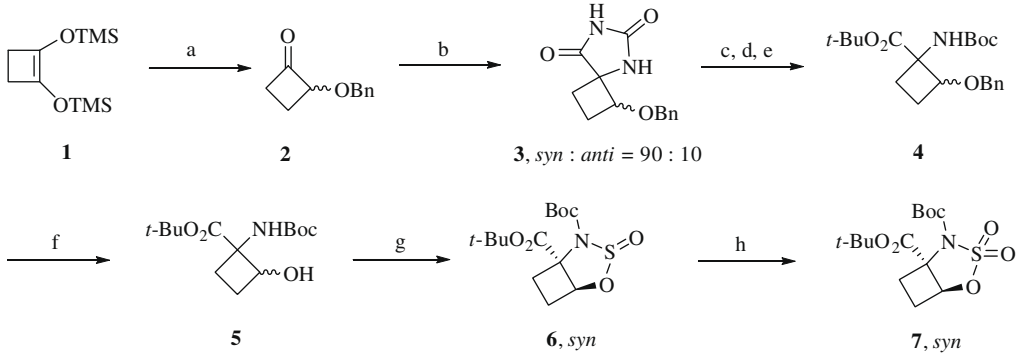
The 2-FACBC cyclic sulfamidate precursor for fluorine-18 radiolabeling was prepared in a series of synthetic steps starting from 1,2-bis(trimethylsiloxy)cyclobtene (**1**), which is shown in Scheme 1. The compound 1,2-bis(trimethylsiloxy)cyclobtene was treated with benzyl alcohol in an acidic media[36] to afford 2-(benzyloxy)cyclobutanone (**2**) in 62% yield. The hydantoins (**3**) were prepared through modified Strecker synthesis in a 9:1 mixture of *syn*- to *anti*-isomers as determined by ^{1}H NMR. This mixture **3** was hydrolyzed in aqueous base solution to give *syn*- and *anti*-2-benzyloxy-cyclobutane amino acids followed by the protection of amino and carboxylic groups in the forms of *N*-Boc and *tert*-butyl ester to afford *syn*- and *anti*-*tert*-butyl 2-(benzyloxy)-1-(*tert*-butoxycarbonylamino)cyclobutanecarboxylates (**4**) in 35% yield from **2**. Catalytic debenzylation using palladium on active carbon released *syn*- and *anti*-alcohols (**5**) in quantitative yield. In the key synthetic step, reaction of **5** with thionyl chloride in polar solvent acetonitrile[37] gave exclusive *syn*-isomer of cyclic sulfamidite (**6**) in 80% yield. The oxidation of **6** with sodium periodate provided *syn*-isomer of cyclic sulfamidate (**7**) in 96% yield as *anti*-2-[^{18}F]FACBC labeling precursor.

Radiofluorinated *anti*-2-[^{18}F]FACBC **9** was prepared using no-carrier-added (NCA) cyclotron-produced [^{18}F]fluoride and K_{222} followed by hydrolysis with 4 N hydrochloric acid.[38] *anti*-2-[^{18}F]FACBC **9** was obtained by ion-retardation resin chromatographic purification[31,39] (Scheme 2). The procedure required approximately 85 min with decay-corrected yields (DCY) of 29.5 ± 5.4% (n = 5) with radiochemical purity over 99% as measured by radiometric TLC. In a representative synthesis, a total of 103 mCi of [^{18}F]**9** at end of synthesis (EOS) was obtained from 445 mCi of [^{18}F]fluoride at end of bombardment (EOB) using 1 mg (3 μmol) of cyclic sulfamidate precursor *syn*-**7**. While the specific activity of [^{18}F]**9** was not determined directly, the maximum amount of unlabeled material in the final product arising from the precursor is about 1 mg in each case. On the basis of 100 mCi yield at EOS, the amount of nonradioactive material in the final dose is approximately 10 μg per mCi. This amount is comparable to the amount of nonradioactive material present in doses of [^{18}F]FDG and *anti*-[^{18}F]FACBC which arises from the triflate precursors.[29,40] On the basis of the average dosage of 5–20 μCi of [^{18}F]**9** per animal in the in vivo study, the maximum amount of unlabeled material arising from the precursor associated with [^{18}F]**9** per injection was approximately 0.05–0.2 μg. The major byproduct arising from the labeling precursor is expected to consist of *anti*-1-amino-2-hydroxy-cyclobutyl-1-carboxylic acid arising from competing nucleophilic reaction at C-2 by water or hydroxide ions.

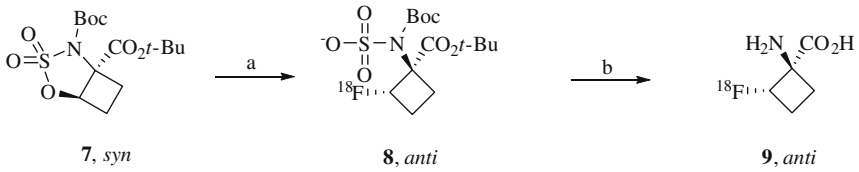
The non-radioactive *anti*-2-FACBC **9** was prepared from the *syn*-alcohol **5**, which was reacted with (diethylamino)sulfur trifluoride (DAST)[41,42] followed by acid hydrolysis using trifluoroacetic acid (TFA) as shown in Scheme 3.

The in vitro studies were performed in 9L rat gliosarcoma cells in Hank's Balanced Salt Solution (HBSS) incubated for 30 min at 37 °C with or without inhibitors to evaluate the compound tumor cell uptake profile and transport mechanism. 10 mM of 2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid (BCH), 10 mM of *N*-methyl-α-aminoisobutyric acid (MeAIB) and the combination of 10 mM of alanine-cysteine-serine (ACS, 3.3 mM of each amino acid) were used as system L, system A, and system ASC amino acid transport inhibitors, respectively. The results of the amino acid uptake assays are depicted in Figure 2. In the absence of inhibitors, *anti*-2-[^{18}F]FACBC **9** showed good level of intracellular accumulation, 3.3 ± 0.1% of the initial dose per 0.5 million cells (%ID/5×10^5 cells) in 9L gliosarcoma cells. ACS inhibited 73% of the uptake compared to control (p <0.0001, one-way ANOVA). In the presence of BCH, 59% of inhibition was observed compared to control (p = 0.0001, one-way ANOVA). Interestingly, 33% uptake inhibition occurred with MeAIB relative to control (p = 0.0012, one-way ANOVA). These results demonstrate that compound **9** enter 9L gliosarcoma cells in vitro involves both system L and non-system L transport, likely system A.

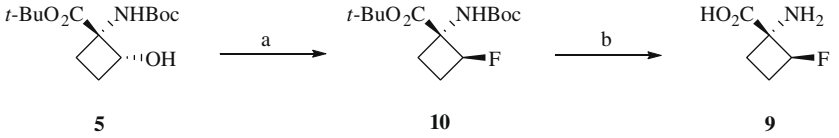
The in vivo biodistribution studies were performed in male Fischer 344 rats with 9L tumors implanted intracranially. The radioactivity in tumors and in normal tissues of tumor-bearing rats (n = 4 each time point) was calculated at 15, 30, 60, and 120 min post-injection (pi) and normalized as percent injected dose per gram tissue (%ID/g). The uptake of radioactivity of *anti*-2-[^{18}F]FACBC **9** in tumor and brain is presented in Figure 3. The experiments showed that this amino acid had rapid and prolonged accumulation in tumors, in the values of 0.51, 0.66, 0.65, and 0.60%ID/g at 15, 30, 60, and 120 min pi, respectively, and was significantly high-



Scheme 1. Synthesis of *anti*-2-[^{18}F]FACBC cyclic sulfamidate precursor **7**. Reagents and conditions: (a) BnOH, HCl, Et_2O, 0–80 °C; (b) $(NH_4)_2CO_3$, NH_4Cl, KCN, EtOH/H_2O, 70 °C; (c) NaOH (aq), 120 °C; (d) $(Boc)_2O$, Et_3N, CH_3OH, rt; (e) $Cl_3CC(–NH)OC(CH_3)_3$, rt; (f) H_2, Pd–C (cat.), rt; (g) $SOCl_2$, pyridine, CH_3CN, −40 °C; (h) NaIO4, $RuCl_3$ (cat.), CH_3CN, H_2O, 0 °C to rt.



Scheme 2. Radiosynthesis of *anti*-2-[^{18}F]FACBC **9**. Reagents and conditions: (a) [^{18}F]KF, K_{222}, 90 °C, 10 min; (b) 4 N HCl, 110 °C, 10 min.



Scheme 3. Synthesis of *anti*-2-FACBC **9**. Reagents and conditions: (a) DAST, DCM, −70 °C to rt, overnight; (b) TFA, DCM, rt.

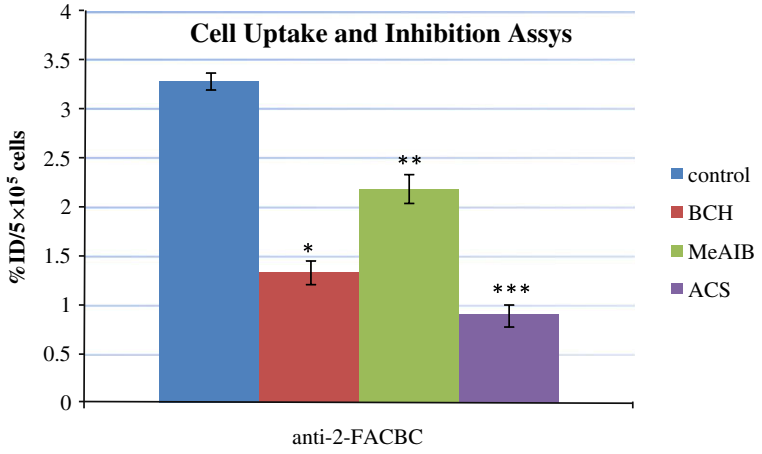


Figure 2. 9L Cell uptake and inhibition assays with [^{18}F]**9**. Uptake is normalized as the initial dose per 0.5 million cells (%ID/5 × 10^5 cells). Error bars indicate ±standard deviation (n = 3). * = 59% reduction versus control, p = 0.0001. ** = 33% reduction versus control, p = 0.0012. *** = 73% reduction versus control, p <0.0001.

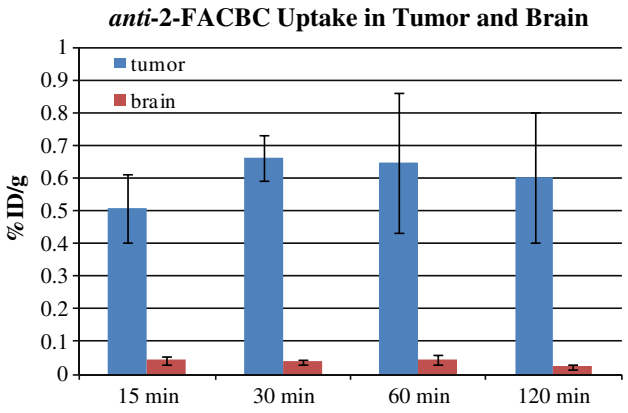


Figure 3. Radioactivity uptake in tumor and brain of 9L tumor-bearing Fisher rats with *anti*-2-[^{18}F]FACBC **9**. Uptake is normalized as percent injected dose per gram tissue (%ID/g). Error bars indicate ±standard deviation (n = 4 at each time point). p <0.0001 (at all time points, tumor vs brain, one-way ANOVA).

er than in normal brain tissue (p <0.0001 at all time points, one-way ANOVA). The uptake in normal brain tissue was less than 0.04%ID/g at all time points thus the tumor to normal brain uptake ratios were 12:1, 17:1, 14:1, and 26:1 at 15, 30, 60, and 120 min pi, respectively, for *anti*-2-[^{18}F]FACBC **9**. Low uptake was found in blood, heart, liver, lung, muscle, and bone. The low bone uptake with this radiotracer indicates that [^{18}F]**9** was stable and free [^{18}F]fluoride was not generated during the time course of the study. The tumor to brain ratios of [^{18}F] labeled *anti*-FACBC[30], *syn*-FACBC[31] *syn*-FMACBC[32] and *anti*-FMACBC[32] at 60 min pi were 6.6:1, 7.6:1, 6.9:1, and 8.9:1, respectively, in the same animal model. Thus, the candidate compound **9** is comparable with *anti/syn*-FACBC and *anti/syn*-FMACBC for the good tumor to brain ratios. The remarkable difference of this amino acid compared with its analogues FACBC and FMACBC was that *anti*-2-[^{18}F]FACBC **9** entered 9L tumors through certain degree of non-system L amino acid transport, possibly system A transport, as demonstrated in cell inhibition study. One of representative system A substrates is amino acid 2-amino-3-fluoro-2-methylpropanoic acid (FAMP).[13,37] Amino acids 2-FACBC **9** and FAMP possess amazing similar structures as shown in Figure 4. It may not be surprised that these two compounds share similar in vitro and in vivo properties since the chemical structure of a compound is a major factor to regulate the selectivity of the transport systems. The system A transport is inactive at the luminal surface of normal endothelium of the

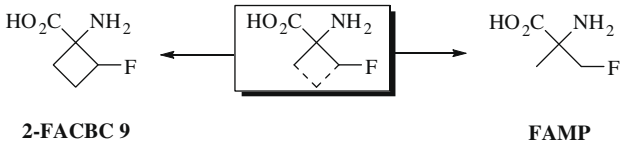


Figure 4. The chemical structures of 2-FACBC **9** and FAMP.

blood–brain barrier (BBB),[13] which might explain very low normal brain uptake of *anti*-2-[^{18}F]FACBC **9**. The higher tumor to brain ratio of compound [^{18}F]**9** compared to *anti*- and *syn*-[^{18}F]FACBC and *anti*- and *syn*-[^{18}F]FMACBC was contributed to the lower normal brain uptake.

In summary, a new non-natural cyclobutyl amino acid, *anti*-2-[^{18}F]FACBC **9** has been synthesized and biologically evaluated. This compound demonstrated moderate levels of tumor uptake in vitro and in vivo in a 9L rat gliosarcoma brain tumor model and it is an amino acid transporter substrate. These results are comparable with those of *anti*- and *syn*-FACBC and *anti*- and *syn*-FMACBC in the same animal model, which support the candidacy of *anti*-2-[^{18}F]FACBC **9** as a promising PET brain tumor imaging agent.

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