Bioorganic & Medicinal Chemistry 17 (2009) 1982–1990

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Stereoselective synthesis and biological evaluation of *syn*-1-amino-3-[^{18}F]-fluorocyclobutyl-1-carboxylic acid as a potential positron emission tomography brain tumor imaging agent

Weiping Yu [a], Larry Williams [a], Vernon M. Camp [a], Eugene Malveaux [a], Jeffrey J. Olson [b], Mark M. Goodman [a,*]

[a] Department of Radiology, School of Medicine, Emory University, Atlanta, GA 30322, USA
[b] Department of Neurosurgery, School of Medicine, Emory University, Atlanta, GA 30322, USA

ARTICLE INFO

Article history:
Received 21 November 2008
Revised 14 January 2009
Accepted 15 January 2009
Available online 21 January 2009

Keywords:
syn-FACBC
Fluorine-18
Amino acid transporter
PET

ABSTRACT

Amino acid *syn*-1-amino-3-fluoro-cyclobutyl-1-carboxylic acid (*syn*-FACBC) **12**, the isomer of *anti*-FACBC, has been selectively synthesized and [^{18}F] radiofluorinated in 52% decay-corrected yield using no-carrier-added [^{18}F]fluoride. The key step in the synthesis of the desired isomer involved stereoselective reduction using lithium alkylborohydride/zinc chloride, which improved the ratio of *anti*-alcohol to *syn*-alcohol from 17:83 to 97:3. *syn*-FACBC **12** entered rat 9L gliosarcoma cells primarily via L-type amino acid transport in vitro with high uptake of 16% injected dose per 5×10^5 cells. Biodistribution studies in rats with 9L gliosarcoma brain tumors demonstrated high tumor to brain ratio of 12:1 at 30 min post injection. In this model, amino acid *syn*-[^{18}F]FACBC **12** is a promising metabolically based radiotracer for positron emission tomography brain tumor imaging.

1. Introduction

The development of radiotracers that accumulate preferentially in tumor cells has been one of the active radiopharmaceutical interests. A number of classes of compounds have been investigated, including metabolically based radiotracers such as amino acids, carbohydrates, and nucleosides. The application of radiolabeled amino acids using positron emission tomography (PET) to detect brain tumors has received considerable attention due to potential advantages over other imaging modalities. Conventional imaging methods such as computed tomography (CT) and magnetic resonance imaging (MRI) do not always reliably distinguish residual and recurring tumor from tissue injury so they are not optimal for monitoring the effectiveness of surgical treatment, or for detecting tumor recurrence. The leading PET agent [^{18}F]FDG, a glucose derivative, shows high uptake in normal brain cortical tissue as well as in inflammatory tissues associated with radiation necrosis which can complicate the diagnostic evaluation of brain tumors.[1] Many tumor cells have increased amino acid transport relative to normal cells as well as to inflammatory tissues associated largely with the utilization of nutrients in tumors. In contrast to [^{18}F]FDG, the uptake of amino acids in macrophages and other inflammatory cells is low.[2] This may complement [^{18}F]FDG for grading and staging tumors.[3]

Many amino acids have been radiolabeled to study potential imaging characteristics.[3–18] These radiolabeled amino acids differ in ease of synthesis, in vivo biodistribution and formation of metabolites. Amino acids enter cells mainly from two pathways, from outside by carrier mediated transporters or are derived from intracellular protein recycling. For radiotracers used for tumor imaging, the former pathway is the major consideration. Naturally occurring amino acids and their closely related derivatives, represented by L-[^{11}C-methyl]methionine (L-[^{11}C]MET),[12] L-1-[^{11}C]-tyrosine (L-[^{11}C]TYR)[5,15] and [^{18}F]fluoro-L-phenylalanine,[19] enter and accumulate in tumor cells not only by transporters but also by protein synthesis processes. These amino acid radiotracers are susceptible to in vivo metabolism through multiple pathways, giving rise to numerous radiolabeled metabolites. Thus, graphical analysis with the necessary accuracy for reliable measurement of tumor metabolic activity is not possible. The shortcomings associated with naturally occurring amino acids may be overcome with non-natural amino acids. Non-natural amino acids are expected to have certain degree of metabolic stability. They may not be effectively incorporated into proteins. These properties can potentially simplify the analysis of tracers' in vivo kinetics and tumor cells uptake mechanisms.

A number of [^{18}F] labeled non-natural cyclobutyl amino acids have been prepared in our lab for PET tumor imaging, including

* Corresponding author. Tel.: +1 404 727 9366; fax: +1 404 727 3488.
E-mail address: mgoodma@emory.edu (M.M. Goodman).

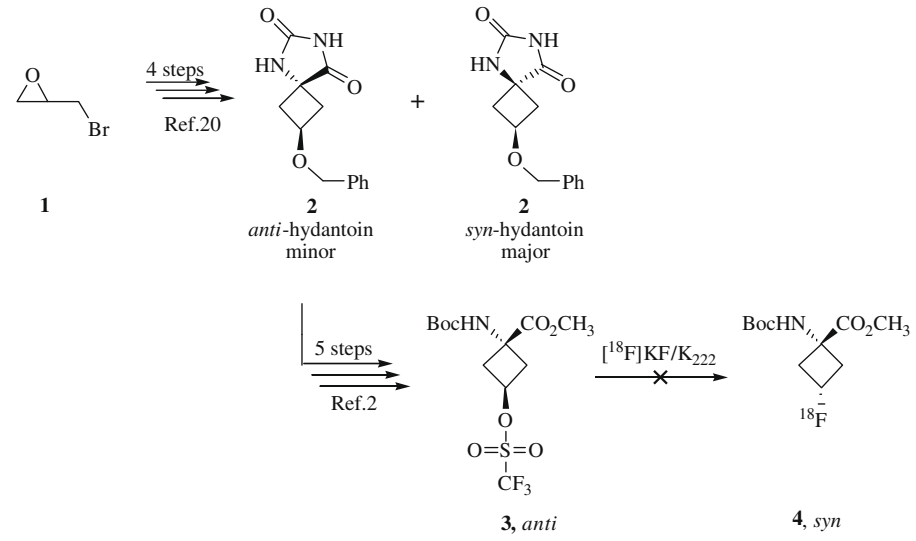
0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.01.032

anti-1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid (*anti*-[^{18}F]FACBC)[1,20] and *syn*- and *anti*-1-amino-3-[^{18}F]fluoromethyl-cyclobutane-1-carboxylic acid (*syn*- and *anti*-[^{18}F]FMACBC).[21] These compounds showed high tumor uptake in 9L rat gliosarcoma tumors. Among these amino acids, the PET tracer *anti*-[^{18}F]FACBC is undergoing human preclinical trials to validate it as a valuable imaging agent for the diagnosis and management of treatment of cancer.[22,23] The method for preparation of [^{18}F]FACBC reported previously[20] affords only gram quantities of the precursor, cis (*syn*)-1-(*tert*-butoxycarbonylamino)-3-(trifluoromethylsulfonyloxy)cyclobutane-1-carboxylic methyl ester, for producing *anti*-isomer of [^{18}F]FACBC. Since the preparation of the precursor of *syn*-[^{18}F]FACBC, trans (*anti*)-1-carbamate-3-(trifluoromethylsulfonyloxy)cyclobutane-1-carboxylic methyl ester involved the separation of the minor isomer from the major isomer in a 87:13 mixture of intermediates (**2**), gram scale synthesis was impractical. The work presented here demonstrated the general synthetic route to produce gram quantities of *syn*-[^{18}F]FACBC precursor (**10d**), radiosynthesis and the biological evaluation of *syn*-[^{18}F]FACBC **12** with 9L rat gliosarcoma tumor model.

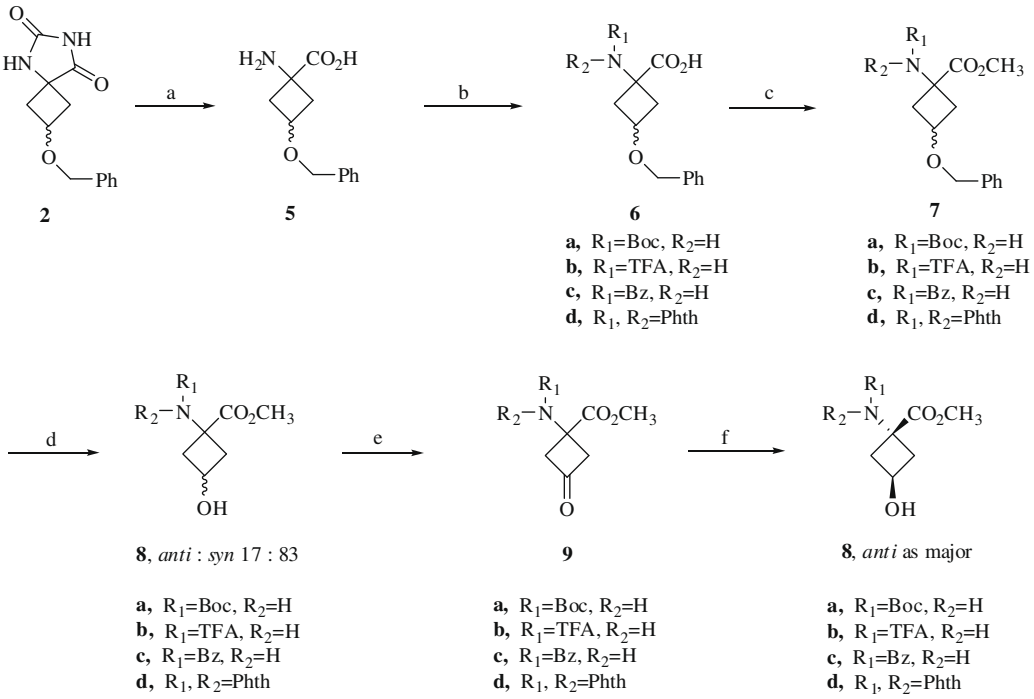
2. Results and discussion

2.1. Chemistry

The *anti*-triflate precursor of *syn*-FACBC for radiolabeling was synthesized in a series of synthetic steps starting from epi-



Scheme 1. Synthesis of *syn*- and *anti*-hydantoin **2** and *anti*-triflate **3**.

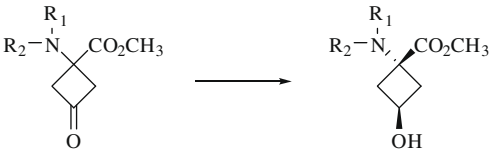


Scheme 2. Synthesis of stereoselective *anti*-alcohol **8**. Reagents and conditions: (a) 3 N NaOH, 120 °C, overnight; (b) di-*tert*-butyl dicarbonate (**6a**); trifluoroacetic anhydride (**6b**); benzoyl chloride (**6c**); phthalic anhydride (**6d**); (c) $(CH_3)_3SiCHN_2$, rt, 30 min; (d) H_2, 10% Pd–C, overnight; (e) NMO, TPAP, rt, 5 h; (f) reductant A–E (see Table 1). Notes: Boc = *tert*-butoxycarbonyl; TFA = trifluoroacetyl; Bz = benzoyl; Phth = phthaloyl.

bromohydrin (**1**) and benzyl bromide. The key intermediates, a 83:17 mixture of *syn*/*anti*-5-(3-benzyloxycyclobutane)hydantoins (**2**) was prepared in four steps as described previously.[20] The separation of the desired *anti*-hydantoin by chromatography using reported procedure was time consuming and impractical. Even though we managed to synthesize *anti*-triflate (**3**) as the precursor for *syn*-[^{18}F]FACBC, the radiofluorination was unsuccessful, due part to the bulky Boc group which hindered $^{18}F^-$ to access reaction center at C-3 (Scheme 1).

Our approach to solve the problems involved: (1) using less bulky amino protecting groups or cyclic imide such as phthalimide instead of Boc so that the C-3 is more exposed for nucleophilic attacking by ^{18}F anion and (2) selectively synthesizing *anti*-precursor at the later synthetic stage by using stereoselective reagent to avoid tedious chromatographic separation procedure. The detailed syntheses are depicted in Scheme 2. The *N*-Boc derivatives were included for comparison with the other compounds.

Table 1



9a, R_1=Boc, R_2=H
9b, R_1=TFA, R_2=H
9c, R_1=Bz, R_2=H
9d, R_1, R_2=Phth

8a, R_1=Boc, R_2=H
8b, R_1=TFA, R_2=H
8c, R_1=Bz, R_2=H
8d, R_1, R_2=Phth

Ketones	Reductants	Time	Yield (%)	Ratio of alcohol **8**	
				anti	*syn*
9a	A	24 h	98	70	30
	A/$ZnCl_3$	24 h	100	70	30
	B	24 h	—		
	B/$ZnCl_3$	24 h	—		
	C	24 h	92	78	22
	C/$ZnCl_3$	24 h	90	80	20
	D	30 min	97	55	45
	D/$ZnCl_3$	30 min	96	60	40
	E	30 min	98	55	45
	E/$ZnCl_3$	30 min	96	55	45
9b	A	24 h	74	80	20
	A/$ZnCl_3$	24 h	93	**95**	**5**
	B	24 h	<0.1	43	57
	B/$ZnCl_3$	24 h	—		
	C	24 h	70	75	25
	C/$ZnCl_3$	24 h	68	78	22
	D	30 min	100	55	45
	D/$ZnCl_3$	30 min	98	55	45
	E	30 min	95	62	38
	E/$ZnCl_3$	30 min	99	55	45
9c	A	24 h	98	75	25
	A/$ZnCl_3$	24 h	77	**93**	**7**
	B	24 h	—		
	B/$ZnCl_3$	24 h	—		
	C	24 h	90	55	45
	C/$ZnCl_3$	24 h	91	60	40
	D	30 min	95	55	45
	D/$ZnCl_3$	30 min	92	58	42
	E	30 min	96	55	45
	E/$ZnCl_3$	30 min	93	55	45
9d	A	17 h	79	82	18
	A/$ZnCl_3$	24 h	79	**97**	**3**
	B	24 h	—		
	B/$ZnCl_3$	24 h	—		
	C	24 h	88	75	25
	C/$ZnCl_3$	24 h	91	82	18
	D	30 min	99	55	45
	D/$ZnCl_3$	30 min	92	60	40
	E	30 min	89	58	42
	E/$ZnCl_3$	30 min	95	60	40

A = L-Selectride®; B = LS-Selectride®; C = $NaBH_4$; D = BH_3–THF/(*R*)-CBS (cat.); E = BH_3–THF/(*S*)-CBS (cat.).

The 83:17 mixture of *syn*/*anti*-hydantoins (**2**) was hydrolyzed at 120 °C with 3 N sodium hydroxide (aq) to give *syn*- and *anti*-1-amino-3-benzyloxycyclobutane-1-carboxylic acids (**5**). Subsequent amino protection with (a) di-*tert*-butyl dicarbonate; (b) trifluoroacetic anhydride; (c) benzoyl chloride; and (d) phthalic anhydride, respectively, provided corresponding *syn*/*anti*-carbamates **6a–d**. *syn*/*anti*-1-Carbamate-3-benzyloxy-cyclobutane-1-carboxylic acid methyl esters **7a–d** were prepared by treatment of **6a–d** with (trimethylsilyl)diazomethane,[24] respectively. Debenzylation using hydrogen and palladium (0) catalyst on carbon gave 83:17 mixture of *syn*/*anti*-1-carbamate-3-hydroxy-cyclobutane-1-carboxylic acid methyl esters **8a–d**. Since the precursor for *syn*-FACBC was the *anti*-isomer, which was the minor component from above syntheses, it was difficult to isolate from the major *syn*-compound in large scale by the chromatographic method reported earlier.[20] To optimize the synthetic method, the mixture of *syn*/*anti*-alcohols was firstly oxidized to the corresponding ketone **9a–d** with *N*-methylmorpholine-*N*-oxide (NMO) and tetra-*n*-propylammonium perruthenate (TPAP) in methylene chloride.[25–27] Secondly several reductants have been tested for the selectivity of converting the ketone **9a–d** back to alcohol **8a–d** with the *anti*-alcohols as the major products. The ratios of the *anti*- and *syn*-isomers were determined by ^{1}H NMR and the results are summarized in Table 1.

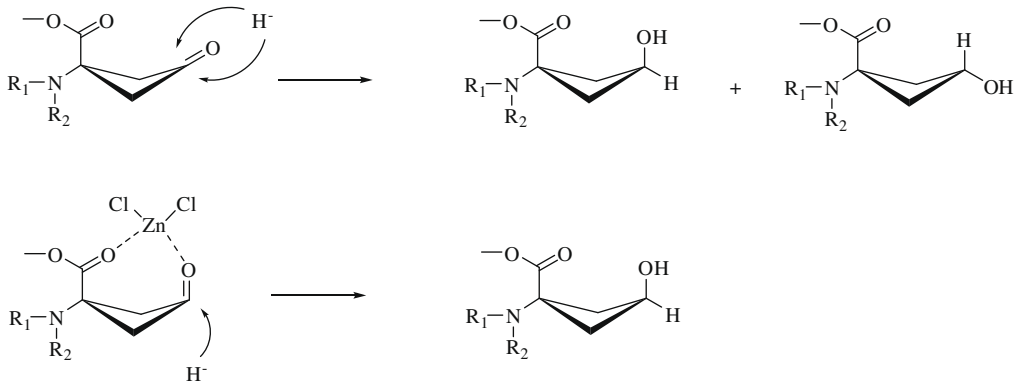
Among the reductants chosen, BH_3-THF/(*S*)- or (*R*)-CBS (oxazaborolidine) catalyst[28] gave little stereoselectivity to all reductive products **8a–d**, although these conditions led to excellent yields. The reductant LS-Selectride® (lithium trisiamylborohydride) failed to provide any products in all attempts, except trace of **8b** was collected. Sodium tetrahydridoborate, with or without zinc chloride, showed same and certain degree of stereoselectivity, which gave 3–4:1 ratios of *anti*- to *syn*-alcohols **8a–d**. However further separation procedures are inevitable. The best stereoselectivie reductant in this study was L-Selectride® (lithium tri-*sec*-butylborohydride) with zinc chloride as a chelating Lewis acid. Hydride attack came generally from both sides of the cyclobutanone but the chelates promoted more effective approach to *anti*-configurational alcohols (Scheme 3).

The *anti*-alcohol **8a–d** were subsequently reacted with triflic anhydride to give *anti*-1-carbamate-3-[(trifluoromethyl)sulfonyl]oxy-cyclobutanecarboxylic acid methyl esters **10a–d** as the radiolabeling precursors, see Scheme 4.

The ^{19}F reference of *syn*-FACBC **12** was prepared from the *anti*-*N*-phthaloyl amino acid methyl ester *anti*-**8d** by treatment of diethylaminosulfur trifluoride (DAST)[29,30] followed by deprotection with hydrazine hydrate as shown in Scheme 5.

2.2. Radiolabeling

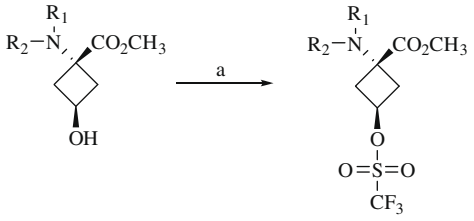
Radiofluorinated *syn*-[^{18}F]FACBC **12** was prepared in two steps using no-carrier-added (NCA) nucleophilic substitution with dried [^{18}F]KF, potassium carbonate and Kryptofix in acetonitrile using the triflate **10d** (Scheme 6). The precursors of *anti*-triflates **10a–c** were attempted and failed to give any ^{18}F-labeled products. The exchange between [^{18}F]fluoride and the leaving group occurred in 10 min at 90 °C. Unreacted [^{18}F]fluoride and any radiolabeled charged by-products were eliminated by passing the reaction mixture through a silica SepPak®. Removal of protecting groups was achieved by using hydrazine hydrate at 75 °C for 10 min. The *syn*-[^{18}F]FACBC **12** was purified by ion-retardation resin chromatography.[24] The procedure required approximately 80 min from the end of bombardment (EOB) with decay-corrected yields (DCY) of 51.5 ± 15.6% (n = 7) in over 95% radiochemical purity as measured by radiometric TLC.



Scheme 3.

2.3. Cell uptake assays

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 compounds tumor cell uptake profile and transport mechanism. 10 mM 2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid (BCH) and 10 mM *N*-methyl-α-aminoisobutyric acid (MeAIB) were used as L- and A-type amino acid transport inhibitors, respectively.[13,31–33] We also used the combination of 10 mM alanine-cysteine-serine (ACS, equal molar amount) as quality control for our inhibition assays, since these amino acids can be used as wide-spectra block agents for amino acid transporters. In the absence of inhibitors, *syn*-[^{18}F]FACBC **12** showed high levels of intracellular accumulation, 15.8 ± 2.4% of the initial dose per 0.5 million cells (%ID/5 × 10^5 cells) in 9L gliosarcoma cells. In the presence of BCH, 38.6% of inhibition was observed compared to controls ($p < 0.02$, 1-way ANOVA). On the contrast, no uptake inhibition was detected with MeAIB relative to controls. When ACS was used, up to 77.8% uptake reduction occurred compared to controls ($p < 0.001$, 1-way ANOVA). For comparison, we carried out the same cell assays for compound *anti*-FACBC under the identical condition. *anti*-FACBC showed a very similar cell uptake profile to that of *syn*-FACBC. Without inhibitors, its cell uptake was 12.3 ± 0.4%ID/5 × 10^5 cells. The cell uptake was reduced to 2.9 ± 0.6%ID/5 × 10^5 cells ($p < 0.0001$, 1-way ANOVA) and 0.18 ± 0.02%ID/5 × 10^5 cells ($p < 0.0001$, 1-way ANOVA) by BCH and by ACS, respectively. The inhibition by MeAIB (6%) was not significant. These results, which are depicted in Figure 1, demonstrate that both compounds *syn*-FACBC **12** and *anti*-FACBC are selective substrates for L-type amino acid transporter in 9L gliosarcoma cells in vitro.



8

a, R_1=Boc, R_2=H
b, R_1=TFA, R_2=H
c, R_1=Bz, R_2=H
d, R_1, R_2=Phth

10

a, R_1=Boc, R_2=H (=**3**)
b, R_1=TFA, R_2=H
c, R_1=Bz, R_2=H
d, R_1, R_2=Phth

Scheme 4. Syntheses precursors of *anti*-triflates **10a–d**. Reagents and conditions: (a) Tf_2O, Py, DCM, 0 °C, 15 min.

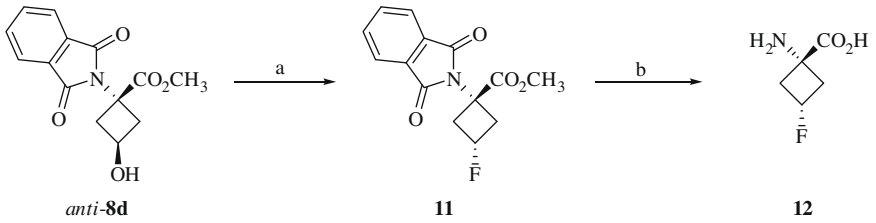
2.4. In vivo biodistribution studies in tumor-bearing rats

The in vivo biodistribution studies were performed in Fischer rats with 9L tumors implanted intracranially. Based on the research results we published earlier,[1,3,16,21] the tissue distribution of radioactivity after tail vein injection of amino acid tracers in the normal tissues of tumor-bearing animals was similar to that seen in the normal animals of this model. As the result, we only used tumor-bearing rats for measuring radioactivity biodistrubution in this study. 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 (*p.i.*) and normalized as percent injected dose per gram tissue (%ID/g). The uptake of radioactivity of *syn*-[^{18}F]FACBC **12** in tumor and in other tissues is presented in Table 2.

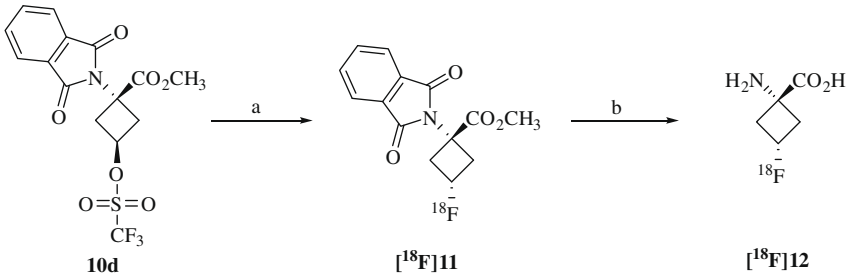
The experiments showed that this amino acid had rapid and prolonged accumulation in tumors, ranged from 1.4–2.1%ID/g and was significantly higher than in normal brain tissue ($p < 0.05$ at all time points, one-way ANOVA). The uptake in normal brain tissue was less than 0.25%ID/g at all time points thus the tumor to normal brain uptake ratios were in the range of 7.2:1–11.5:1. Except in pancreas, uptake in other tissues tested was lower than that in tumor ($p < 0.02$, two-way ANOVA). The low bone uptake of radioactivity with this radiotracer indicates that compound **12** is metabolically stable so free fluoride was not generated during the time course of the study. The analogs *anti*-FACBC, *syn*-FMACBC and *anti*-FMACBC, showed uptake of radioactivity in tumor at 60 min *p.i.* of 1.72, 1.59 and 2.50%ID/g in the same animal model, respectively, which resulted in tumor to brain ratios of 6.6:1, 6.9:1 and 8.9:1, respectively.[1,21] Thus, compound **12** is comparable to *anti*-FACBC, *syn*-FMACBC and *anti*-FMACBC, in the terms that all these amino acids enter 9L cells via L-type transport system in vitro and show high levels of radioactivity uptake in 9L tumor in vivo in this animal model.

3. Conclusions

A new PET tumor imaging ligand, *syn*-FACBC **12** has been selectively synthesized in gram quantities, [^{18}F] labeled in high radiochemical yield (>50% DCY) and high radiochemical purity, and biologically evaluated in rodent 9L gliosarcoma brain tumor model. The compound demonstrated high levels of tumor uptake in vitro and in vivo with good tumor to brain ratios ranging from 7:1 to 12:1. It transported into cells primarily through L-type amino acid transporter with little if any A-type transport property. These results are comparable to its analogues of *anti*-FACBC, *syn*-FMACBC and *anti*-FMACBC in the same animal model, which support the candidacy of *syn*-FACBC **12** as promising PET brain tumor imaging agent.



Scheme 5. Synthesis of F-19 reference of *syn*-FACBC **12**. Reagents and conditions: (a) DAST, DCM, −70 °C to rt, overnight; (b) N_2H_4–H_2O, 75 °C, 30 min.



Scheme 6. Radiosynthesis of *syn*-[^{18}F]FACBC **12**. Reagents and conditions: (a) [^{18}F]KF, K_{222}, K_2CO_3, 90 °C, 10 min; (b) N_2H_4–H_2O, 75 °C, 10 min.

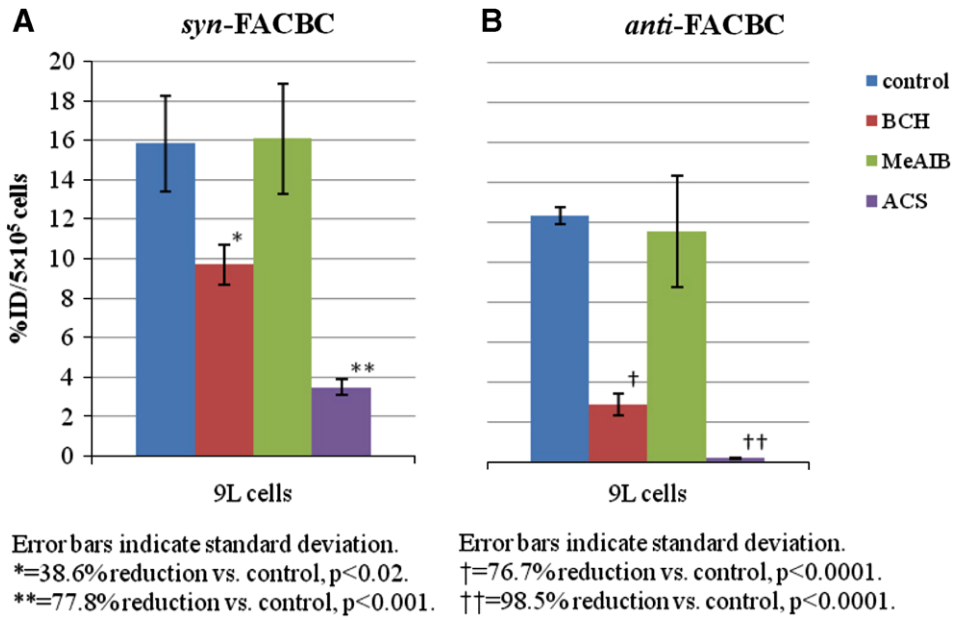


Figure 1. 9L cell uptake and inhibition assays with (A) [^{18}F]**12** and (B) *anti*-[^{18}F]FACBC (%ID/5 × 10^5 cells).

4. Experimental

4.1. Chemistry

All chemicals, solvents and materials used were obtained from commercially available sources and used without purification. Chemicals were purchased from Aldrich Chemicals Co. (Milwaukee, WI USA) and Sigma Chemical Co. (St. Louis, MO USA), or otherwise indicated, and solvents were purchased from Aldrich Chemicals and VWR Scientific Products (West Chester, PA USA). Thin-layer chromatography (TLC) analyses were performed with 250 μm UV254 silica gel backing on aluminum plates (Whatman Ltd.; Maidstone, Kent England). Flash chromatography was carried out using Merck Kieselgel silica gel 60 (230–400 mesh). Melting points were measured in capillary tubes using a Mel-Temp II apparatus (Laboratory Devices, Inc., Holliston, MA USA) and are uncorrected. ^{1}H NMR spectra were recorded on Varian 400 MHz or 300 MHz spectrometers at NMR Center at Emory University, and chemical shifts (δ values) were reported as parts per million (ppm) downfield from tetramethylsilane (TMS). Elemental analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA USA) and were within ±0.4% of the theoretical values. Mass Spectra were done on JEOL JMS-SX102/SX102A/E or VG 70-S double focusing mass spectrometers at Mass Spectroscopy Center at Emory University using high-resolution electrospray ionization (ESI).

Table 2
Biodistribution of radioactivity in tissues of 9L tumor-bearing Fischer rats following intravenous administration of *syn*-[^{18}F]**12**

Tissue	15 min	30 min	60 min	120 min
Blood	0.36 ± 0.08	0.37 ± 0.10	0.32 ± 0.02	0.26 ± 0.04
Heart	0.43 ± 0.09	0.47 ± 0.15	0.38 ± 0.02	0.30 ± 0.05
Lung	0.40 ± 0.13	0.57 ± 0.22	0.42 ± 0.04	0.48 ± 0.05
Liver	0.73 ± 0.18	0.60 ± 0.22	0.41 ± 0.03	0.40 ± 0.10
Pancreas	2.37 ± 0.96	3.36±0.24	2.83 ± 0.59	2.78 ± 0.48
Spleen	0.68 ± 0.18	0.61 ± 0.12	0.52 ± 0.03	0.40 ± 0.06
Kidney	0.76 ± 0.16	0.80 ± 0.18	0.70 ± 0.02	0.56 ± 0.08
Muscle	0.35±0.16	0.43 ± 0.002	0.42 ± 0.01	0.39 ± 0.06
Brain	0.20 ± 0.14*	0.18 ± 0.02**	0.20±0.01†	0.25 ± 0.03*
Tumor	1.40 ± 0.49*	2.10 ± 0.96**	1.48 ± 0.09†	1.93 ± 0.72*
Bone	0.25 ± 0.12	0.26 ± 0.07	0.28 ± 0.05	0.20±0.03
Testis	0.17 ± 0.06	0.27 ± 0.03	0.27 ± 0.02	0.21±0.03
Tumor to brain ratio	7.2	11.5	7.6	7.7

Values are reported as mean percent injected dose per gram tissue (%ID/g) ± standard deviation; $n = 4$ at each time point; p values were calculated using one-way ANOVA; *$p < 0.02$, **$p < 0.05$, †$p < 0.002$.

4.2. *syn/anti*-1-[*N*-(*tert*-Butoxycarbonyl)amino]-3-benzyloxy-cyclobutanecarboxylic acid (6a)

syn/anti-1-Amino-3-benzyloxy-cyclobutanecarboxylic acid (**5**) was prepared according to the method described earlier.[20] To a suspension of **5** (2.0 g, 9 mmol) in 50 mL of 9:1 methanol/triethylamine (v/v) was added a 1.5 equiv portion of di-*tert*-butyl dicarbonate (14 g), and the solution was stirred at room temperature overnight. The solvent was removed under reduced pressure and the resulting residue was re-dissolved in 50 mL of water. The pH of the solution was adjusted to 2 with 3 N HCl and the aqueous phase was extracted with ethyl acetate (2 × 50 mL). The combined organic layers were washed with water (1 × 50 mL), dried over magnesium sulfate (anhyd). The *N*-Boc acid **6a** (2.1 g, 69%) was obtained as a white solid suitable for use in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (9H, s, $C(CH_3)_3$), 2.31 (*syn*-, 83%) and 2.50 (*anti*-, 17%) (2H, m, CH_2), 2.64 (*anti*-, 17%) and 2.93 (*syn*-, 83%) (2H, m, CH_2), 4.20 (1H, m, CH), 4.45 (2H, s, OCH_2Ph), 5.14 (1H, br s, NH), 7.27–7.37 (5H, m, aromatic).

4.3. *syn/anti*-1-[*N*-(Trifluoroacetyl)amino]-3-benzyloxy-cyclobutanecarboxylic acid (6b)

To a suspension of *syn/anti*-1-amino-3-benzyloxy-cyclobutanecarboxylic acid (**5**)[20] (1 g, 4.5 mmol) in 30 mL of dichloromethane was added triethylamine (5 equiv). The mixture was cooled to −15 °C and trifluoroacetic anhydride (3 equiv) was added dropwise under an argon atmosphere. The mixture was warmed up to room temperature and stirred overnight. Ammonium chloride (1 M, 10 mL) was added to the mixture and two phases separated after stirred for 15 min. The organic layer was retained, and the aqueous phase was extracted with ethyl acetate (2 × 30 mL). The combined organic layers were washed with water (2 × 30 mL), dried over magnesium sulfate (anhyd). The crude *N*-TFA acid **6b** was purified using silica gel chromatography (30% ethyl acetate in hexanes) to provide **6b** as a clear oil, 1.11 g (82%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.47–2.52 (*syn*-, 83%) and 2.71–2.76 (*anti*, 17%) (2H, m, CH_2), 2.85–2.90 (*anti*, 17%) and 3.00–3.05 (*syn*-, 83%) (2H, m, CH_2), 4.27 (*syn*-, 83%) and 4.36 (*anti*, 17%) (1H, m, CH), 4.47 (*syn*-, 83%) and 4.50 (*anti*, 17%) (2H, s, OCH_2Ph), 5.34 (1H, br s, NH), 7.30–7.37 (5H, m, aromatic).

4.4. *syn/anti*-1-[*N*-(Benzoyl)amino]-3-benzyloxy-cyclobutanecarboxylic acid (6c)

To a suspension of *syn/anti*-1-amino-3-benzyloxy-cyclobutanecarboxylic acid (**5**)[20] (1.14 g, 5.2 mmol) in 50 mL of 2 N sodium hydroxide was added benzoyl chloride (1.2 equiv) at 0 °C over 15 min. The mixture was stirred at 0 °C for 2 h then at room temperature overnight. Ethyl acetate (40 mL) was added to the mixture and the aqueous layer was adjusted to pH 1–2 with hydrochloric acid (concd). The organic phase was retained; the aqueous phase was saturated with sodium chloride and extracted with ethyl acetate (4 × 40 mL). The combined organic phases were dried over sodium sulfate (anhyd) and the solvent was removed under reduced pressure. The crude *N*-Bz acid **6c**, 1.22 g (73%), was used in next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54 (*syn*-, 83%) and 2.80 (*anti*, 17%) (2H, m, CH_2), 3.01 (*anti*, 17%) and 3.22 (*syn*-, 83%) (2H, m, CH_2), 4.28 (1H, m, CH), 4.49 (2H, s, OCH_2Ph), 6.86 (1H, br s, NH), 7.32–8.14 (10H, m, aromatic).

4.5. *syn/anti*-1-[*N*-(Phthaloyl)amino]-3-benzyloxy-cyclobutanecarboxylic acid (6d)

To a suspension of *syn/anti*-1-amino-3-benzyloxy-cyclobutanecarboxylic acid (**5**)[20] (669 mg, 3.03 mmol) in 15 mL of toluene was added phthalic anhydride (2 equiv) and triethylamine (2 equiv). The mixture was heated to reflux at 120 °C for 5 h. After cooled to room temperature, 10 mL of water was added and 3 N HCl was used to adjust the pH of the aqueous solution to 2. The aqueous phase was separated, saturated with sodium chloride and extracted with ethyl acetate (2 × 15 mL). The combined organic phases were dried over sodium sulfate (anhyd), filtered and concentrated to dryness. The crude *N*-Phth acid **6d** was used in next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.81 (*syn*-, 83%) and 2.93 (*anti*, 17%) (2H, m, CH_2), 3.32 (*syn*-, 83%) and 3.56 (*anti*, 17%) (2H, m, CH_2) 4.45 (1H, m, CH), 4.48 (2H, s, OCH_2Ph), 7.32–7.85 (9H, m, aromatic).

4.6. *syn/anti*-1-Carbamate-3-benzyloxy-cyclobutanecarboxylic acid methyl ester (7a–d)

Compound **6a–d** was dissolved in 15 mL of 4:1 benzene/methanol (v/v), respectively, and (trimethylsilyl) diazomethane (2.0 M in hexanes, 1.2 equiv) was added.[24] After stirred at room temperature for 30 min, the solvent was removed under reduced pressure. The crude **7a–d** was purified using silica gel chromatography (20% ethyl acetate in hexanes).

4.6.1. *syn/anti*-1-[*N*-(*tert*-Butoxycarbonyl)amino]-3-benzyloxy-cyclobutanecarboxylic acid methyl ester (7a)

White crystalline solid, 2.0 g from 2.0 g (9.1 mmol) of **5** (66%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (9H, s, $C(CH_3)_3$), 2.31 (*syn*-, 83%) and 2.50 (*anti*-, 13%) (2H, m, CH_2), 2.64 (*anti*-, 13%) and 2.93 (*syn*-, 83%) (2H, m, CH_2), 3.76 (3H, s, OCH_3), 4.12–4.27 (1H, m, CH), 4.45 (2H, s, OCH_2Ph), 5.14 (1H, br s, NH), 7.29–7.38 (5H, m, aromatic).

4.6.2. *syn/anti*-1-[*N*-(Trifluoroacetyl)amino]-3-benzyloxy-cyclobutanecarboxylic acid methyl ester (7b)

Oil, 1.44 g from 1.17 g (3.7 mmol) of **6b** (92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.48–2.53 (*syn*-, 83%) and 2.65–2.70 (*anti*-, 17%) (2H, br m, CH_2), 2.75–2.80 (*anti*-, 17%) and 2.95–3.00 (*syn*-, 83%) (2H, m, CH_2), 3.77 (*syn*-, 83%) and 3.78 (*anti*-, 17%) (3H, s, OCH_3), 4.23–4.29 (1H, m, CH), 4.45 (*anti*-, 17%) and 2.46 (*syn*-, 83%) (2H, s, OCH_2Ph), 7.06 (1H, br s, NH), 7.30–7.37 (5H, m, aromatic).

4.6.3. *syn/anti*-1-[*N*-(Benzoyl)amino]-3-benzyloxy-cyclobutanecarboxylic acid methyl ester (7c)

Clear oil, 1.0 g from 1.14 g (5.2 mmol) of **5** (58%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54 (*syn*-, 83%) and 2.80 (*anti*, 17%)

(2H, m, CH_2), 3.01 (*anti*, 17%) and 3.22 (*syn*-, 83%) (2H, m, CH_2), 3.83 (*anti*-, 17%) and 3.85 (*syn*-, 83%) (3H, s, OCH_3), 4.30 (1H, m, CH), 4.51 (2H, s, OCH_2Ph), 6.66 (1H, br s, NH), 7.30–8.11 (10H, m, aromatic).

4.6.4. *syn/anti*-1-[*N*-(Phthaloyl)amino]-3-benzyloxy-cyclobutanecarboxylic acid methyl ester (7d)

Clear oil, 723 mg from 669 mg (3.0 mmol) of **5** (65%, 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.78–2.83 (*syn*-, 83%) and 2.91–2.96 (*anti*, 17%) (2H, m, CH_2), 3.29–3.34 (*syn*-, 83%) and 3.54–3.59 (*anti*, 17%) (2H, m, CH_2), 3.73 (*syn*-, 83%) and 3.74 (*anti*, 17%) (3H, s, OCH_3), 4.43–4.67 (1H, m, CH), 4.48 (2H, s, OCH_2Ph), 7.27–7.34 (5H, m, aromatic), 7.71–7.73 and 7.82–7.84 (4H, m, aromatic). HRMS, *m/z*, Calcd for $C_{21}H_{20}NO_5$ $[M+H]^+$, 366.1342. Found, 366.1363. Anal. Calcd for ($C_{21}H_{19}NO_5$): C, 69.03; H, 5.24; N, 3.83. Found: C, 68.64; H, 5.28; N, 3.86.

4.7. *syn/anti*-1-Carbamate-3-hydroxy-cyclobutanecarboxylic acid methyl ester (8a–d)

To a solution of **7a**–**d** in 5–10 mL of methanol, respectively, was added 40% (w/w) of 10% palladium on carbon. The reaction mixture was stirred overnight at room temperature under a hydrogen atmosphere. The suspension was then filtered over Celite and concentrated under reduced pressure. Purification via silica gel column chromatography (30% ethyl acetate in hexane) provided the alcohol **8a**–**d.**

4.7.1. *syn/anti*-1-[*N*-(*tert*-Butoxycarbonyl)amino]-3-hydroxy-cyclobutanecarboxylic acid methyl ester (8a)

White crystalline solid, 968 mg from 1389 mg (4.1 mmol) of **7a** (95%), mp 128–131 °C (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (*anti*-, 17%) and 1.45 (*syn*-, 83%) (9H, s, $C(CH_3)_3$), 2.48 (*anti*-, 17%) and 2.56 (*syn*-, 83%) (2H, m, CH_2), 3.00 (*syn*-, 83%) and 3.35 (*anti*-, 17%) (2H, m, CH_2), 3.77 (*syn*-, 83%) and 3.79 (*anti*-, 17%) (3H, s, OCH_3), 4.30 (*syn*-, 83%) and 4.46 (*anti*-, 17%) (1H, m, CH), 5.63 (1H, br s, NH).

4.7.2. *syn/anti*-1-[*N*-(Trifluoroacetyl)amino]-3-hydroxy-cyclobutanecarboxylic acid methyl ester (8b)

Clear oil, 1.0 g from 1.39 g (4.2 mmol) of **7b** (99%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.69–2.74 (*anti*-, 17%) and 2.75–2.80 (*syn*-, 83%) (2H, m, CH_2), 2.89–2.94 (*anti*-, 17%) and 3.01–3.11 (*syn*-, 83%) (2H, m, CH_2), 3.86 (*anti*-, 17%) and 3.88 (*syn*-, 83%) (3H, s, OCH_3), 4.36–4.42 (*syn*-, 83%) and 4.62–4.68 (*anti*-, 17%) (1H, m, CH), 7.47 (1H, br s, NH).

4.7.3. *syn*/*anti*-1-[*N*-(Benzoyl)amino]-3-hydroxy-cyclobutanecarboxylic acid methyl ester (8c)

Clear oil, 550 mg from 980 mg (2.9 mmol) of **7c** (76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.57 (*syn*-, 83%) and 2.73 (*anti*, 17%) (2H, m, CH_2), 2.87 (*anti*, 17%) and 3.13 (*syn*-, 83%) (2H, m, CH_2), 3.81 (*anti*-, 17%) and 3.87 (*syn*-, 83%) (3H, s, OCH_3), 4.37 (*syn*-, 83%) and 4.59 (*anti*, 17%) (1H, m, CH), 6.69 (1H, br s, NH), 7.44–7.83 (5H, m, aromatic).

4.7.4. *syn*/*anti*-1-[*N*-(Phthaloyl)amino]-3-hydroxy-cyclobutanecarboxylic acid methyl ester (8d)

Semi solid, 74 mg from 186 mg (0.51 mmol) of **7d** (53%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.68–2.76 (*syn*-, 83%) and 2.82–2.89 (*anti*-, 17%) (2H, m, CH_2), 3.31–3.42 (2H, m,CH_2), 3.73 (*syn*-, 83%) and 3.76 (*anti*-, 17%) (1H, s, OCH_3), 4.38–4.45 (*anti*-, 17%) and 4.66–4.76 (*syn*-, 83%) (1H, m, CH), 7.71–7.76 (2H, m, aromatic), 7.81–7.86 (2H, m, aromatic). HRMS, *m/z*, Calcd for $C_{14}H_{14}NO_5$ $[M+H]^+$, 276.0873, found, 276.0849.

4.8. 1-Carbamate-3-oxo-cyclobutanecarboxylic acid methyl ester (9a–d)

syn/anti-1-Carbamate-3-hydroxy-cyclobutanecarboxylic acid methyl ester **8a**–**d** in 5 mL of 10% acetonitrile in dichloromethane containing *N*-methylmorpholine *N*-oxide (1.5 equiv) and 4 Å molecular sieves (500 mg/1 mmol, powder), respectively, was stirred for 10 min at 22 °C under argon and then treated with tetra-*n*-propylammonium perruthenate (10% molar equiv). The resulting solution was stirred for 5 h. The solvent was removed and the residue was purified by flash column chromatography (30% ethyl acetate in hexanes) to afford the ketone **9a**–**d**, respectively.

4.8.1. 1-[*N*-(*tert*-Butoxycarbonyl)amino)amino]-3-oxo-cyclobutanecarboxylic acid methyl ester (9a)

White solid, 162 mg from 183 mg (0.75 mmol) of **8a** (89%), mp 117–119 °C (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (9H, s, $C(CH_3)_3$), 3.50–3.66 (4H, m, $2CH_2$), 3.84 (3H, s, OCH_3), 5.48 (1H, br s, NH). Anal. Calcd for ($C_{11}H_{17}NO_5$): C, 54.31; H, 7.04; N, 5.76. Found: C, 54.50; H, 6.96; N, 5.61.[20]

4.8.2. 1-[*N*-(Trifluoroacetyl)amino]-3-oxo-cyclobutanecarboxylic acid methyl ester (9b)

Semi solid, 90 mg from 114 mg (0.47 mmol) of **8b** (80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.65–3.93 (4H, m, $2CH_2$), 3.92 (3H, s, OCH_3), 7.50 (1H, br s, NH). HRMS, *m/z*, Calcd for $C_8H_{12}F_3N_2O_4$ $[M+NH_4]^+$, 257.07437, found, 257.07437.

4.8.3. 1-[*N*-(Benzoyl)amino]-3-oxo-cyclobutanecarboxylic acid methyl ester (9c)

Semi solid, 288 mg from 340 mg (1.4 mmol) of **8c** (85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.67–3.86 (4H, m, $2CH_2$), 3.87 (3H, s, OCH_3), 7.30 (1H, br s, NH), 7.44–8.11 (5H, m, aromatic). HRMS, *m/z*, Calcd for $C_{13}H_{14}NO_4$ $[M+H]^+$, 248.09173, found, 248.09176; *m/z*, Calcd for $C_{13}H_{17}N_2O_4$ $[M+NH_4]^+$, 265.11828, found, 265.11831.

4.8.4. 1-[*N*-(Phthaloyl)amino]-3-oxo-cyclobutanecarboxylic acid methyl ester (9d)

White crystalline solid, 112 mg from 125 mg (0.45 mmol) of **8d** (91%). mp 175–176 °C (ethyl acetate/hexane). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (3H, s, OCH_3), 3.98–4.01 (4H, m, $2CH_2$), 7.78–7.81 (2H, m, aromatic), 7.89–7.92 (2H, m, aromatic). HRMS, *m/z*, Calcd for $C_{14}H_{12}NO_5$ $[M+H]^+$, 274.07100, found, 274.0114. Anal. ($C_{14}H_{11}NO_5$) C, H, N.

4.9. *anti*-1-Carbamate-3-hydroxy-cyclobutanecarboxylic acid methyl ester (*anti*-8a–d)

To the solution of the ketone 9a**–**d in 2 mL of THF (anhyd), respectively, was added $ZnCl_2$ (2 equiv, in THF) at room temperature under argon. The solution was stirred at room temperature for 30 min followed by the addition of the L-Selectride® (1.5 equiv) at −78 °C. The mixture was stirred at −78 °C for 2 h then at room temperature overnight. Ammonium chloride (1 N aq, 3 equiv) was added and the mixture was stirred at room temperature for 30 min. The reaction was washed with brine, and aqueous phase was re-extracted with ethyl acetate (2 × 3 mL). The combined organic phases were dried over sodium sulfate (anhyd), filtered and concentrated to dryness. The product was purified by silica gel flash column chromatography using 50% ethyl acetate in hexanes to afford 8a**–**d (*anti*-alcohol as major product, see Table 1).

4.9.1. *anti*-1-[*N*-(*tert*-Butoxycarbonyl)amino]-3-hydroxy-cyclobutanecarboxylic acid methyl ester (*anti*-8a)

White solid, 16 mg from 16 mg (0.066 mmol) of **9a** (100%), mp 108–110 °C (ethyl acetate/hexane, 109–110 °C[20]). ^{1}H NMR

(400 MHz, $CDCl_3$) δ 1.44 (9H, s, $C(CH_3)_3$), 2.53–2.63 (4H, m, $2CH_2$), 3.77 (3H, s, OCH_3), 4.43–4.50 (1H, m, CH), 5.02 (1H, br s, NH). ^{13}C NMR (100 MHz, CD_3OD) δ 28.83, 42.50, 43.07, 53.05, 63.22, 80.71, 158.04, 175.35. Anal. Calcd for ($C_{11}H_{19}NO_5$): C, 53.87; H, 7.81; N, 5.71. Found: C, 53.61; H, 7.79; N, 5.51.[20]

4.9.2. *anti*-1-[*N*-(Trifluoroacetyl)amino]-3-hydroxy-cyclobutanecarboxylic acid methyl ester (*anti*-8b)

Semi solid, 181 mg from 194 mg (0.81 mmol mmol) of **9b** (93%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.69–2.74 (2H, m, CH_2), 2.89–2.94 (2H, m, CH_2), 3.85 (3H, s, OCH_3), 4.62–4.68 (1H, m, CH), 7.02 (1H, br s, NH). ^{19}F NMR (376 MHz, $CDCl_3$) δ −76.43 (s, CF_3). HRMS, *m/z*, Calcd for $C_8H_{10}F_3{}^{39}KNO_4$ $[M+K]^+$, 280.00370, found, 280.00372.

4.9.3. *anti*-1-[*N*-(Benzoyl)amino]-3-hydroxy-cyclobutanecarboxylic acid methyl ester (*anti*-8c)

Semi solid, 44 mg from 57 mg (0.23 mmol mmol) of **9c** (77%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.61–2.66 (2H, m, CH_2), 2.71–2.77 (2H, m, CH_2), 3.69 (3H, s, OCH_3), 4.44–4.51 (1H, m, CH), 6.69 (1H, br s, NH), 7.33–7.77 (5H, m, aromatic).

4.9.4. *anti*-1-[*N*-(Phthaloyl)amino]-3-hydroxy-cyclobutanecarboxylic acid methyl ester (*anti*-8d)

White solid, 65 mg from 82 mg (0.30 mmol) of **9d** (79%), mp 70–72 °C (ethyl acetate/hexane). ^1H NMR (400 MHz, $CDCl_3$) δ 2.82–2.89 (2H, m,CH_2), 3.35–3.42 (2H, m,CH_2), 3.75 (1H, s, OCH_3), 4.38–4.45 (1H, m, CH), 7.73–7.76 (2H, m, aromatic), 7.82–7.86 (2H, m, aromatic). HRMS, *m/z*, Calcd for $C_{14}H_{14}NO_5$ $[M+H]^+$, 276.08665, found, 276.08698. Anal. ($C_{14}H_{13}NO_5$) C, H, N.

4.10. *anti*-1-Carbamate-3-(trifluoromethylsulfonyloxy) cyclobutanecarboxylic acid methyl ester (10a–d)

anti-Alcohol 8a–d was dissolved in 1 mL of dichloromethane, respectively, and cooled to 0 °C under argon. Pyridine (15–20 equiv) and triflic anhydride (5 equiv) were added successively. The mixture was stirred at 0 °C for 15 min and the solvent was removed under reduced pressure. The mixture was purified by flash chromatography (silica, 30% ethyl acetate in hexanes) to afford the *anti*-triflate **10a–d**, respectively.

4.10.1. *anti*-1-[*N*-(*tert*-Butoxycarbonyl)amino]-3-(trifluoromethylsulfonyloxy) cyclobutanecarboxylic acid methyl ester (10a, 3)

Oil, 8 mg from 18 mg (0.072 mmol mmol) of ***anti*-8a** (28%). ^1H NMR (400 MHz, $CDCl_3$) δ 1.44 (9H, s, $C(CH_3)_3$), 3.06 (4H, m, $2CH_2$), 3.80 (3H, s, $COCH_3$), 5.31 (1H, br s, NH), 5.38 (1H, m, CH). ^{19}F NMR (376 MHz, $CDCl_3$) δ −75.64 (s, SCF_3).

4.10.2. *anti*-1-[*N*-(Trifluoroacetyl)amino]-3-(trifluoromethylsulfonyloxy) cyclobutanecarboxylic acid methyl ester (10b)

Semi solid, 18 mg from 19 mg (0.079 mmol) of ***anti*-8b** (61%). ^1H NMR (400 MHz, $CDCl_3$) δ 3.12–3.25 (4H, m, $2 \times CH_2$), 3.94 (3H, s, $COCH_3$), 5.76–5.81 (1H, m, CH), 7.22 (1H, br s, NH). ^{19}F NMR (376 MHz, $CDCl_3$) δ −76.44 (s, $COCF_3$) and −75.49 (s, SCF_3).

4.10.3. *anti*-1-[*N*-(Benzoyl)amino]-3-(trifluoromethylsulfonyloxy) cyclobutanecarboxylic acid methyl ester (10c)

Oil, 8.2 mg from 13.5 mg (0.054 mmol) of ***anti*-8c** (40%). ^1H NMR (400 MHz, $CDCl_3$) δ 2.60–2.85 (4H, m, $2CH_2$), 3.84 (3H, s, OCH_3), 5.26 (1H, m, CH), 7.33 (1H, br s, NH), 7.50–8.10 (5H, m, aromatic). ^{19}F NMR (376MHz, $CDCl_3$) δ −75.52 (s, SCF_3).

4.10.4. *anti*-1-[*N*-(Phthaloyl)amino]-3-(trifluoromethylsulfonyloxy) cyclobutanecarboxylic acid methyl ester (10d)

Clear oil, 15 mg from 11 mg (0.04 mmol) of ***anti*-8d** (93%). ^1H NMR (400 MHz, $CDCl_3$) δ 3.32–3.37 (2H, m, CH_2), 3.83–3.89 (2H, m, CH_2), 3.80 (3H, s, $COCH_3$), 5.40 (1H, m, CH), 7.78–7.80 (2H, m, aromatic), 7.88–7.90 (2H, m, aromatic). ^{19}F NMR (376MHz, $CDCl_3$) δ −75.64 (s, SCF_3).

4.11. *syn*-1-[*N*-(Phthaloyl)amino]-3-fluoro-cyclobutanecarboxylic acid methyl ester (11)

anti-Alcohol **8d** (50 mg, 0.18 mmol) was dissolved in 2 mL of dichloromethane and diethylaminosulfur trifluoride (DAST, 3 equiv, in 2 mL of dichloromethane) was added at −78 °C under argon. The mixture was allowed to warm to room temperature and stirred overnight. The organic layer was washed with water and brine, dried with sodium sulfate (anhyd), filtered and concentrated under reduced pressure. Purification of the crude product by flash chromatography (silica, 20% ethyl acetate in hexanes) yielded compound **11** as a clear oil (24 mg, 48%). ^1H NMR ($CDCl_3$) δ 2.93–3.04 (2H, m, CH_2), 3.36–3.43 (2H, m, CH_2), 3.73 (3H, s, CH_3), 5.25–5.32 and 5.39–5.46 (1H, d-p, J = 54.8, 6.8 Hz, CHF), 7.73–7.76 (2H, m, aromatic), 7.82–7.86 (2H, m, aromatic). ^{19}F NMR (376 MHz, $CDCl_3$), δ −162.775 to −162.454 (m, CHF). HRMS, *m/z*, Calcd for $C_{14}H_{13}FNO_4$ $[M+H]^+$, 278.08231, found, 278.08249. Anal. Calcd for ($C_{14}H_{12}FNO_4$), C, 60.65; H, 4.36; N, 5.05. Found: C, 60.38; H, 4.40; N, 5.10.

4.12. *syn*-1-*N*-Amino-3-fluoro-cyclobutanecarboxylic acid (12)

Compound **11** (17 mg, 0.06 mmol) in 300 μL of hydrazine hydrate was stirred at 75 °C for 30 min. After cooled to room temperature, the mixture was neutralized to pH 7 with 4 N hydrochloric acid and passed successively through an ion-retardation resin column (AG 11A8 50–100 mesh, BioRad, Hercules, CA, USA), an alumina N Sep-Pak® and an HLB Sep-Pak® (Waters, Inc., Milford, MA USA) with water. The fractions containing *syn*-FACBC **12** were collected and dried to give a white solid (4.6 mg, 58%), mp 215 °C (decomposition). ^1H NMR (D_2O) δ 2.74–2.84 (2H, m, CH_2), 3.16–3.21 (2H, m, CH_2), 5.20–5.28 and 5.32–5.40 (1H, d-p, J = 56.4, 6.6 Hz, CHF). Anal. Calcd for ($C_5H_8FNO_2$): C, 45.11; H, 6.06; N, 10.52. Found: C, 45.02; H, 6.24; N, 10.68.

4.13. Radiosynthesis

[^{18}F]HF was produced at Emory University with a Siemens 11 MeV RDS 112 negative-ion cyclotron (Knoxville, TN, USA) by the ^{18}O (p, n) ^{18}F reaction using [^{18}O]H_2O (95%). A fully automated synthesis system developed for the Siemens computer programmable chemistry process control unit (CPCU) was used for the preparation of [^{18}F]**12**. The CPCU is a valve-and-tubing system which is designed to accommodate two glass reaction vessels and a number of reagent and solvent reservoir containers. Briefly, the automated production of [^{18}F]**12** was performed by reacting 8 mg (20 μmol) of triflate precursor **10d** in 1 mL of acetonitrile with 600 mCi NCA [^{18}F]fluoride in the presence of Kryptofix 222 (5 mg, 13.3 μmol) and potassium carbonate (1 mg, 7.2 μmol) at 90 °C for 10 min. After passing a silica SepPak® (Waters, Inc.), the product was hydrolyzed with 200 μL of hydrazine hydrate at 75 °C for 10 min then neutralized with 4 N hydrochloric acid. The radiolabeled amino acid [^{18}F]**12** was purified by passing the resulting solution successively through an ion-retardation resin (BioRad) column, an alumina N Sep-Pak®, an HLB Sep-Pak® (Waters), and a 0.2 μm Gelman teflon filter (VWR) with sterile saline into a dose vial, which was ready for use in in vitro and in vivo studies. The isolated radio-

chemical yields were determined using a dose-calibrator (Capintec CRC-712M). Thin layer chromatograms of the radiolabeled compounds were analyzed with a Raytest System (model: Rita Star. Germany) using the same type of silica TLC plates from Whatman. The identity of the radiolabeled product [^{18}F]**12** was confirmed by comparing the R_f value of the radioactive product visualized with radiometric TLC with the R_f value of the authentic non-radioactive form of the compound **12** visualized with ninhydrin stain (R_f = 0.4, 4:1:1 CH_3CN/H_2O/MeOH).

4.14. Amino acid uptake and inhibition assays

The amino acid uptake assays were performed with cultured rat 9L gliosarcoma cells as described previously.[3,21] In summary, The 9L cell line was cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum, 100 units/mL penicillin and 100 μg/mL streptomycin. Cells were maintained in T-150 tissue culture flasks under humidified incubator conditions (37 °C, 5% CO_2/95% air) and were routinely passaged at confluence. For the cell uptake experiment, 9L cells were dispersed with a 0.05% solution of Trypsin/EDTA, washed with Hank's balanced salt solution (HBSS) twice and were adjusted to a final concentration of 5×10^7 cells/mL in HBSS.

In this study, approximately 5×10^5 cells were exposed to 5 μCi of [^{18}F]-**12** in amino acid free HBSS with or without transport inhibitors (10 mM BCH, 10 mM MeAIB or 10 mM ACS) for 30 min under incubating conditions in 1.5 mL conical tubes. Each assay condition was performed in triplicates. After incubation, cells were twice centrifuged (75 G for 5 min) and rinsed with ice-cold amino-acid/serum-free HBSS to remove residual activity in the supernatant. The activity in tubes was counted in a Packard Cobra II Auto-Gamma counter, the raw counts decay corrected, and the activity per cell number determined. The data from these studies was normalized as percent uptake relative to standard per 5×10^5 cells. The cell uptake data were analyzed with 1-way ANOVA using ProStat (Poly Software International, Pearl River, NY).

4.15. Rodent biodistribution in tumor-bearing rats

All animal experiments were carried out under humane conditions and were approved by the Institutional Animal Use and Care Committee (IUCAC) and Radiation Safety Committee at Emory University. Cultured 9L cells were implanted into the brains of male Fischer 344 rats (125–175 g) for biodistribution studies as described previously.[1,3,21] Briefly, following anesthesia rats were placed in a stereotactic head holder and were injected intracranially with 5 μL suspension of rat 9L gliosarcoma cells (5×10^4 cells per rat). Brain tumors progression could be determined by monitoring weight loss, apathy and hunched posture in the tumor-bearing rats. A total of 16 tumor-bearing animals were used at 10–12 days after implantation in the tissue distribution study. Intravenous doses of 15–30 μCi of [^{18}F]**12** were administered in 0.1–0.3 mL of sterile saline. Groups of 4 rats were sacrificed at 15, 30, 60 and 120 min post injection of the tracer. The animals were dissected, and selected tissues were weighed and counted along with dose standards in a Packard Cobra II Auto-Gamma Counter. The raw counts were decay corrected, and the counts were normalized as the percent of total injected dose per gram of tissue (%ID/g). The uptake of activity in the tissues and tumors was recorded and analyzed with one-way and two-way ANOVA using ProStat software.

Acknowledgements

We are grateful to Dr. Bing Wang of the NMR Center at Emory University for his assistance with NMR studies and Zhaobin Zhang for his assistance with the biological studies. Research supported by Nihon Medi-Physics Co., Ltd.

References and notes

1. Shoup, T. M.; Olson, J.; Hoffman, J. M.; Votaw, J.; Eshima, D.; Eshima, L.; Camp, V. M.; Stabin, M.; Votaw, D.; Goodman, M. M. *J. Nucl. Med.* **1999**, *40*, 331.
2. Oka, S.; Hattori, R.; Kurosaki, F.; Toyama, M.; Williams, L. A.; Yu, W.; Votaw, J. R.; Yoshida, Y.; Goodman, M. M.; Ito, O. *J. Nucl. Med.* **2007**, *48*, 46.
3. McConathy, J.; Martarello, L.; Malveaux, E. J.; Camp, V. M.; Simpson, N. E.; Simpson, C. P.; Bowers, G. D.; Olson, J. J.; Goodman, M. M. *J. Med. Chem.* **2002**, *45*, 2240.
4. Biersack, H. J.; Coenen, H. H.; Stocklin, G.; Reichmann, K.; Bockisch, A.; Oehr, P.; Kashab, M.; Rollmann, O. *J. Nucl. Med.* **1989**, *30*, 110.
5. Daemen, B. J. G.; Elsinga, P. H.; Paans, A. M.; Lemstra, W.; Konings, A. W.; Vaalburg, W. *Int. J. Radiat. Appl. Instrum. Part B, Nucl. Med. Biol.* **1991**, *18*, 503.
6. Dunzendorfer, U.; Schmall, B.; Bigler, R. E.; Zanzonico, P. B.; Conti, P. S.; Dahl, J. R.; Kleinert, E.; Whitmore, W. F. *Eur. J. Nucl. Med.* **1981**, *6*, 535.
7. Hatazawa, J.; Ishiwata, K.; Itoh, M.; Kameyama, M.; Kubota, K.; Ido, T.; Matsuzawa, T.; Yoshimoto, T.; Watanuki, S.; Seo, S. *J. Nucl. Med.* **1990**, *30*, 1809.
8. Ishiwata, K.; Hatazawa, J.; Kubota, K.; Kameyama, M.; Itoh, M.; Matsuzawa, T.; Takahashi, T.; Iwata, R.; Ido, T. *Eur. J. Nucl. Med.* **1989**, *15*, 665.
9. Jager, P. L.; Vaalburg, W.; Pruim, J.; de Vries, E. G.; Langen, K. J.; Piers, D. A. *J. Nucl. Med.* **2001**, *42*, 432.
10. Kubota, K.; Ishiwata, K.; Kubota, R.; Yamada, S.; Takahashi, J.; Abe, J.; Fukuda, H.; Ido, T. *J. Nucl. Med.* **1996**, *37*, 320.
11. Langen, K. J.; Hamacher, K.; Weckesser, M.; Floeth, F.; Stoffels, G.; Bauer, D.; Coenen, H. H.; Pauleit, D. *Nucl. Med. Biol.* **2006**, *33*, 287.
12. Langstrom, B.; Antoni, G.; Gullberg, P.; Halldin, C.; Malmborg, P.; Nagren, K.; Rimland, A.; Svard, H. *J. Nucl. Med.* **1987**, *28*, 1037.
13. Laverman, P.; Boerman, O. C.; Corstens, F. H.; Oyen, W. J. *Eur. J. Nucl. Med.* **2002**, *29*, 681.
14. Lilja, A.; Bergstrom, K.; Hartvig, P.; Spannare, B.; Halldin, C.; Lundqvist, H.; Langstrom, B. *Am. J. Neuroradiol.* **1985**, *6*, 505.
15. Luurtsema, G.; Medema, J.; Elsinga, P. H.; Visser, G. M.; Vaalburg, W. *Appl. Radiat. Isot.* **1994**, *45*, 821.
16. McConathy, J.; Martarello, L.; Malveaux, E. J.; Camp, V. M.; Simpson, N. E.; Simpson, C. P.; Bowers, G. D.; Zhang, Z.; Olson, J. J.; Goodman, M. M. *Nucl. Med. Biol.* **2003**, *30*, 477.
17. Sutinen, E.; Jyrkkio, S.; Gronroos, T.; Haaparanta, M.; Lehikoinen, P.; Nagren, K. *Eur. J. Nucl. Med.* **2001**, *28*, 847.
18. Wester, H. J.; Herz, M.; Weber, W.; Heiss, P.; Senekowitsch-Schmidtke, R.; Schwaiger, M.; Stocklin, G. *J. Nucl. Med.* **1999**, *40*, 205.
19. Kubota, K.; Ishiwata, K.; Kubota, R.; Yamada, S.; Takahashi, J.; Abe, Y.; Fukuda, H.; Ido, T. *J. Nucl. Med.* **1996**, *37*, 320.
20. McConathy, J.; Voll, R. J.; Yu, W.; Crowe, R. J.; Goodman, M. M. *Appl. Radiat. Isot.* **2003**, *58*, 657.
21. Martarello, L.; McConathy, J.; Camp, V. M.; Malveaux, E. J.; Simpson, N. E.; Simpson, C. P.; Olson, J. J.; Bowers, G. D.; Goodman, M. M. *J. Med. Chem.* **2002**, *45*, 2250.
22. Nye, J. A.; Schuster, D. M.; Yu, W.; Camp, V. M.; Goodman, M. M.; Votaw, J. R. *J. Nucl. Med.* **2007**, *48*, 1017.
23. Schuster, D. M.; Votaw, J. R.; Nieh, P. T.; Yu, W.; Nye, J. A.; Master, V.; Bowman, F. D.; Issa, M. M.; Goodman, M. M. *J. Nucl. Med.* **2007**, *48*, 56.
24. Yu, W.; McConathy, J.; Olson, J. J.; Camp, V. M.; Goodman, M. M. *J. Med. Chem.* **2007**, *50*, 6718.
25. Griffith, W. P.; Ley, S. V.; Whitcombe, G. P.; White, A. D. *J. Chem. Soc., Chem. Commun.* **1987**, *21*, 1625.
26. Ley, S. V.; Norman, J.; Griffith, W. P.; Marsden, S. P. *Synthesis* **1994**, *7*, 639.
27. Meltzer, P. C.; Wang, B.; Chen, Z.; Blundell, P.; Jayaraman, M.; Gonzalez, M. D.; George, C.; Madras, B. K. *J. Med. Chem.* **2001**, *44*, 2619.
28. Corey, E. J.; Helal, C. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 1986.
29. Ferreira, S. B. *Synlett* **2006**, *7*, 1130.
30. Golubev, A. S.; Schedel, H.; Radics, G.; Fioroni, M.; Thusta, S.; Burger, K. *Tetrahedron Lett.* **2004**, *45*, 1445.
31. Palacin, M.; Estevez, R.; Bertran, J.; Zorzano, A. *Physiol. Rev.* **1998**, *78*, 969.
32. Saier, M. H. J.; Daniels, G. A.; Boerner, P.; Lin, J. *J. Membr. Biol.* **1988**, *104*, 1.
33. Shotwell, M. A.; Kilberg, M. S.; Oxender, D. L. *Biochim. Biophys. Acta, Rev. Biomembr.* **1983**, *737*, 267.