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Synthesis and Biological Evaluation of (S)-Amino-2-methyl-4-[⁷⁶Br]bromo-3-(E)-butenoic Acid (BrVAIB) for Brain Tumor Imaging

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Abstract

The novel compound, (S)-amino-2-methyl-4-[⁷⁶Br]-bromo-3-(E)-butenoic acid (BrVAIB, [⁷⁶Br]**5**), was characterized against the known system A tracer, IVAIB ([¹²³I]**8**). [⁷⁶Br]**5** was prepared in a 51% ± 19% radiochemical yield with high radiochemical purity (98%). The biological properties of [⁷⁶Br]**5** were compared with those of [¹²³I]**8**. Results showed that [⁷⁶Br]**5** undergoes mixed amino acid transport by system A and system L transport, while [¹²³I]**8** had less uptake by system L. [⁷⁶Br]**5** demonstrated higher uptake than [¹²³I]**8** in DBT tumors 1 h after injection (3.7 ± 0.4% ID/g vs 1.5 ± 0.3% ID/g) and also showed higher uptake vs [¹²³I]**8** in normal brain. Small animal PET studies with [⁷⁶Br]**5** demonstrated good tumor visualization of intracranial DBTs up to 24 h with clearance from normal tissues. These results indicate that [⁷⁶Br]**5** is a promising PET tracer for brain tumor imaging and lead compound for a mixed system A and system L transport substrate.

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The authors declare no competing financial interest.

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.5b01035. Whole body biodistribution on mice with subcutaneous DBT tumors shown as bar graph representation, uptake values, and uptake ratios (PDF)

Molecular formula strings (CSV)

INTRODUCTION

Research in the past few decades has shown an increased interest in metabolic imaging of cancer with positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging techniques.^{1–4} The most widely utilized PET tracer is the glucose analogue 2-deoxy-2- ^{18}F fluoro-D-glucose (FDG), which is used clinically in a wide range of cancers.^{5,6} However, there are limitations to using ^{18}F FDG such as increased uptake in inflammatory tissues and high physiologic uptake in some normal tissues such as the brain which often makes evaluation of lesions in these locations challenging.⁷ Although the 110 min half-life of ^{18}F allows for batch production and remote distribution, the relatively short half-life does limit the geographic range of distribution and prevents imaging at later time points beyond 4–6 h after injection.

A range of radiolabeled amino acid substrates for amino acid transporters known to be upregulated in many cancer cells have been developed.^{8,9} A variety of radiolabeled amino acids including the system L transport substrates L- ^{11}C methionine (MET),^{8,10,11} O-(2- ^{18}F fluoroethyl)-L-tyrosine (FET),^{12–15} and 6- ^{18}F fluoro-3,4-dihydroxy-L-phenylalanine (FDOPA),^{16,17} the system A amino acid transport substrates α - ^{11}C -aminoisobutyric acid (AIB)¹⁸ and α - ^{11}C -methylaminoisobutyric acid (MeAIB),^{18,19} and the system ASCT2 and system L substrate *anti*-1-amino-3- ^{18}F -fluorocyclobutyl-1-carboxylic acid (FACBC)²⁰ have been used extensively for oncologic imaging in both preclinical and human studies. Another group of amino acid derivatives target the metabolic pathway, glutaminolysis, as well as the amino acid transporter, system x_c.^{21,22} Compounds such as ^{18}F -(2*S*,4*R*)-4-fluoroglutamine,^{23–25} (4*S*)-4-(3- ^{18}F -fluoropropyl)-L-glutamate (BAY 94-9392),^{24,26–28} and L-[5- ^{11}C]glutamine²⁹ have also been used in preclinical and clinical studies. Delayed imaging may allow better, higher tumor to nontarget tissue uptake ratios due to washout over time from nontarget tissues.^{8,30}

Over 20 amino acid transporter families have been identified, varying by substrate selectivity and biological properties. System L amino acid transporters (LAT1–4), particularly LAT1, have been the major target of amino acid tracer development. System L family members function in a sodium-independent manner with LAT1 and LAT2 acting through an exchange mechanism which couples intracellular influx of amino acids with efflux of intracellular amino acids while LAT3 and LAT4 mediate facilitated diffusion.^{8,30}

L-type transporters preferentially transport neutral, large, branched amino acids, such as leucine, phenylalanine, tyrosine, and tryptophan. System L is active at the blood–brain barrier (BBB) which is desirable for imaging gliomas and other brain tumors with relatively intact BBBs that are not visualized using contrast enhancement on CT or MRI.^{31–33} A number of amino acid based system L substrates have been developed including *O*-(2-[¹⁸F]fluoroethyl)-*L*-tyrosine (FET),^{12–15} 6-[¹⁸F]fluoro-3,4-dihydroxy-*L*-phenylalanine (FDOPA),^{16,17} 3-[(¹²³I)]iodo-*L*- α -methyltyrosine (IMT),³⁴ and 4-[(^{123/124/131}I)]iodo-*L*-phenylalanine (IPA),^{35–37} all of which have been used for imaging of gliomas. In addition to providing distinct metabolic information, these amino acids have less uptake in the normal brain and inflammatory tissues compared to [¹⁸F]FDG. One important limitation of system L substrates is washout over time from tumor tissue due to the reversible nature of system L transport which can lead to lower tumor to normal tissue contrast (i.e., lower tumor to tissue ratios).

System A preferentially transports small, neutral amino acids (such as alanine and glycine). The co-transportation of sodium ions is coupled to the amino acid uptake through this transporter.^{38,39} This influx of amino acids through system A is essentially unidirectional under physiological conditions, allowing concentration of the transported substrates intracellularly and potentially providing favorable characteristics for imaging and therapy.⁹ *N*-methylation of amino acids tends increase selectivity toward A-type transporters, as most other amino acid transporters do not recognize this modification.^{40,41} A number of PET and SPECT tracers targeting system A have been developed including [¹¹C]-2-aminoisobutyric acid (AIB), [¹¹C]-2-(methylamino)isobutyric acid (MeAIB),¹⁹ (*S*)-amino-2-methyl-4-[(¹²³I)]iodo-3-(*E*)-butenoic acid (IVAIB), 2-amino-3-fluoro-2-methylpropanoic acid (FAMP), and 3-fluoro-2-methyl-2-(methylamino)propanoic acid (*N*-MeFAMP).⁴² Overall, system A transport substrates have not been evaluated as extensively as system L substrates for oncologic imaging. Because pure system A transport substrates do not cross the BBB, their use is restricted to brain tumors with disrupted BBBs (unlike system L substrates).

In this study, we describe the synthesis and biological evaluation and comparison of the novel compound (*S*)-amino-2-methyl-4-bromo-3-(*E*)-butenoic acid (BrVAIB, **5**) and the previously reported system A tracer, (*S*)-amino-2-methyl-4-iodo-3-(*E*)-butenoic acid (IVAIB, **8**).⁴² The bromovinyl derivative was selected for evaluation for a variety of reasons. Our primary goal was to determine the differences, if any, in the biological properties of the brominated derivative versus the previously studied iodinated derivative **8**. Additionally, the longer half-life of ⁷⁶Br (16 h) allows for later imaging time points compared to the more commonly used PET isotopes of ¹¹C (*t*_{1/2} = 20.3 min) or ¹⁸F (*t*_{1/2} = 109.8 min). The sister isotope, ⁷⁷Br (*t*_{1/2} = 57.0 h), is suitable for therapy through Auger electron emission and for imaging with γ emissions at 239 keV (23%) and 521 keV (22%).^{43–45} The longer half-life of ⁷⁷Br could also help provide a large dose to tumor cells exhibiting prolonged retention of the radiolabeled amino acid.

In this study, compound **5** was radiolabeled with bromine-76 while IVAIB was radiolabeled with iodine-123. The transport of [⁷⁶Br]**5** into DBT glioma cells was evaluated using amino acid inhibition studies using known system A, L and other small neutral amino acid inhibitors. Biological evaluation of [⁷⁶Br]**5** and [¹²³I]**8** was performed to evaluate the

compounds' potential as molecular imaging agents utilizing biodistribution and imaging studies in a mouse model of high grade glioma.

RESULTS AND DISCUSSION

Chemistry

Synthesis of the nonradiolabeled **5** derivative was accomplished in five steps, illustrated in Scheme 1. The starting carboxylic acid was selectively protected with a *tert*-butyl ester using *N,N*-dimethylformamide di-*tert*-butyl acetal in excess to give the desired ester in a 60% yield, **1**. The primary alcohol was subsequently oxidized to the aldehyde **2** using general Swern oxidation conditions. Initial attempts to generate the geminal dibromoalkene **3**, using triphenylphosphine (PPh₃) and carbon tetrabromide (CBr₄), led to poor yields. However, the additional step of overnight stirring of zinc dust with PPh₃ and CBr₄ enhanced the reaction yields to provide modest amounts of compound **3** with average yields of around 42%.^{46,47}

The key synthetic step was the reduction of compound **3** to the vinyl bromide, **4**. Heating the dibromide compound with excess dimethyl phosphate and triethylamine in DMF yielded a 10:1 mixture of the desired (*E*)- and the (*Z*)-vinylic isomers as confirmed by ¹H NMR.⁴⁸ The mixture of isomers could not be readily separated on a normal silica gel column. However, doping the silica gel with silver nitrate as reported in the literature proved successful for complete separation of the (*E*)- and (*Z*)-stereoisomers.⁴⁹ Removal of the protecting groups was accomplished using a mixture of trifluoroacetic acid in dichloromethane to yield the (*E*)-vinyl bromide **5** in quantitative yield.

Synthesis of IVAIB followed the previously published route by Yu and co-workers,⁴² except as with the synthesis of **5**, the methyl ester protecting group was exchanged for the *tert*-butyl protection. Yields of intermediates were nearly identical to the methyl ester derivative, even with the bulkier protecting group. This route was mainly utilized to synthesize the trimethyl tin precursor, **7**, needed for radiolabeling with ⁷⁶Br and ¹²³I, Scheme 2.

Radiosynthesis

Initial attempts at incorporating ⁷⁶Br into the compound yielded mixed results. A freshly prepared mixture of acetic acid and hydrogen peroxide, 4 h prior to labeling, led to maximum labeling efficiencies around 35%, even with longer reaction times. This reaction also resulted in multiple radiolabeled species, as determined by instant thin layer chromatography (iTLC); however, the species were not fully characterized. Another commonly used oxidant, bromoperoxidase, was tried but yielded similar results, with maximum labeling efficiencies around 45%. Attempts at labeling with chloramine T as the oxidizing agent yielded better results, with labeling efficiencies upward of 70%; however, the presence of multiple radiolabeled species discouraged use of this reagent for this labeling.

Ultimately, the optimized radiosynthesis of [⁷⁶Br]**5** was achieved using cyclotron produced ammonium [⁷⁶Br]bromide and commercially available peracetic acid (30%) diluted in acetic

acid. Under these conditions, a 20 min reaction of the tin precursor **7** at room temperature provided high labeling efficiency with an average ^{76}Br incorporation of $76 \pm 14\%$ ($n = 6$), Scheme 3. Overall synthesis, isolation, and purification of $[^{76}\text{Br}]\mathbf{5}$ was completed in approximately 70 min and provided the final product in isolated yields of $51.3 \pm 19.1\%$ (dc, $n = 6$), purity 97%, Figure 1, and specific activity of $>135 \text{ mCi (5.0 GBq)/}\mu\text{mol}$. We did not attempt to optimize the specific activity of $[^{76}\text{Br}]\mathbf{5}$ because amino acid transporters typically have K_m values in μM to low mM ranges, and endogenous amino acids are present in relatively high concentrations. Thus, high specific activity is typically not necessary for imaging with radiolabeled amino acids as has been shown with $[^{18}\text{F}]\text{FDOPA}$ prepared in relatively low specific activity through carrier-added electrophilic $[^{18}\text{F}]\text{fluorination}$.⁵⁰ Modifications to the production of ^{76}Br , reduction of potential sources of nonradioactive bromide in the solutions and materials used in the radiosynthesis starting from larger amounts of ^{76}Br of the final product, and HPLC purification to remove nonradioactive compounds arising from the precursor are possible methods to increase the specific activity.

$[^{123}\text{I}]\text{IVAIB}$, the radioiodinated derivative of **8**, was prepared with $[^{123}\text{I}]\text{sodium iodide}$ under similar conditions reported by Yu et al.⁴² with comparable reaction times, 2 h, yields $67.8 \pm 6.9\%$ (dc, $n = 5$), purity of 98%, and specific activity of $>96 \text{ mCi (3.6 GBq)/}\mu\text{mol}$. The overall radiolabeling with ^{123}I was longer due to intermediate steps of quenching the active peroxide as well as extraction of the radiolabeled compound with an organic solvent followed by evaporation of the solvent. These steps were not carried out with the bromination to reduce the overall hand dose given to the radiochemist during the radiolabeling process.

Tin byproducts and unreacted ^{76}Br or ^{123}I from both syntheses were removed from the desired final product by using an ion-retardation resin packed column in sequence with a C-18 solid phase cartridge. The compounds were eluted in one of two ways. Sterile water was used for all in vitro cell uptake studies to reduce the number of sodium counterions when running sodium-free assays. The compounds had final concentrations 1 mCi (0.037 GBq)/mL and were in a pH range between 6 and 7. For in vivo biodistribution and PET/CT studies, products were diluted into 0.9% saline with final concentrations of approximately 1–1.5 mCi (0.037–0.056 GBq)/mL and a pH of ~6.

Cell Uptake Assays

Competitive amino acid cell uptake studies were performed using DBT glioma cells and known amino acid transporter inhibitors to evaluate the transport systems mediating the cellular uptake of these compounds. The non-natural amino acids *N*-methyl- α -amino isobutyric acid (MeAIB)⁵¹ and (*R,S*)-(*endo,exo*)-2-aminobicyclo(2,2,1)-heptane-2-carboxylic acid (BCH)⁵² are well-characterized competitive inhibitors of system A and system L amino acid transport, respectively. Additionally, a combination of the natural amino acids L-alanine, L-serine, and L-cysteine were used to competitively inhibit a wide range of other neutral amino acid transporters (depicted as ASC).

The assays were performed at 37 °C for a relatively short incubation period (60 s) to allow ample time for sufficient uptake while limiting the potential for efflux by reversible transport system mechanisms such as those in system L and system ASC. Control and BCH

conditions were also performed in the presence and absence of sodium counterion salts. In these sodium-free conditions (depicted as Cho-sucrose and Cho-BCH), the buffer/mediums were prepared with choline salts. Because system L transport does not depend on the concentration of sodium and BCH can inhibit some sodium-dependent amino acid transport systems, the uptake inhibition by BCH in sodium-free conditions is the best measure of system L transport. The results of the in vitro amino acid cell uptakes assays using [^{76}Br]5 and [^{123}I]8 are depicted in Figure 2.

The uptake of [^{76}Br]5 was partially blocked by MeAIB (system A) with $56 \pm 12\%$ uptake relative to the control. In the presence of MeAIB, the uptake of [^{123}I]8 was $25 \pm 3.4\%$ compared to the sodium control condition, indicating substantial transport by system A which was more substantial for [^{123}I]8 than for [^{76}Br]5. This result is consistent with prior uptake studies performed in rat 9L gliosarcoma cells.⁴² The uptake of [^{76}Br]5 in the Na BCH and Na ASC conditions was $43 \pm 8.5\%$ and $25 \pm 6.6\%$ relative to control, respectively, which was very similar to the results obtained with [^{123}I]8 ($39 \pm 12\%$ and $22 \pm 1.6\%$, respectively).

Consistent with predominantly sodium-dependent system A transport, the uptake of [^{123}I]8 in the choline control was reduced to $33 \pm 6.6\%$ compared to the sodium control, and the addition of BCH did not significantly further reduce the uptake of [^{123}I]8 ($31 \pm 12\%$ compared to sodium control), indicating a lack of system L transport. In contrast, the uptake of [^{76}Br]5 in choline control conditions ($46 \pm 10\%$ compared to the sodium control conditions) was significantly further reduced by the addition of BCH (25 ± 4.1 compared to sodium control, $p < 0.05$), consistent with a component of system L transport of [^{76}Br]5. These results indicate that the substitution of bromine ([^{76}Br]5) for iodine ([^{123}I]8) increases the amount of system L transport for this class of tracers. Given that system L preferentially transports large neutral amino acids and the iodo substituent has a larger radius than the bromo substituent, this result is somewhat unexpected and may be related to the greater electronegativity of bromine. Table 1 gives a summary of the amino acid transporter families targeted, an overall summary of their transport characteristics, and a summary of the contribution of the transport systems evaluated in these studies to the uptake of [^{76}Br]5 and [^{123}I]8 by DBT glioma cells.

Biodistribution Studies in Mice with Subcutaneous DBT Gliomas

Mice with subcutaneous DBT flank tumors were used at 14 days after tumor cell implantation. Each mouse was intravenously injected in the tail vein with approximately 56 μCi (2.07 MBq) of [^{76}Br]5 or 45 μCi (1.67 MBq) of [^{123}I]8. Animals were euthanized at 5 min, 30 min, 1 h, 3 h, and 24 h after injection ($n = 4$ for each tracer at each time point). Organs and tissues of interest were harvested, weighed, measured for the amount of activity (decay corrected), and the data were calculated as percent of injected dose per gram (% ID/g). The results with [^{76}Br]5 and [^{123}I]8 are shown in Tables 2 and 3, respectively.

Tumor uptake of [^{76}Br]5 was rapid with $2.5 \pm 0.64\%$ ID/g at 5 min and increased to $3.8 \pm 0.95\%$ ID/g at 30 min after injection. In contrast, the uptake in most normal tissues dropped substantially between the 5 and 30 min time points. Tumor uptake of [^{76}Br]5 was stable at 1

h after injection with progressive washout at 3 and 24 h. After 24 h, the majority of activity had cleared out of organs and tissues, with a small amount of activity remaining in the tumor ($0.25 \pm 0.03\%$ ID/g). The brain uptake of [^{76}Br]5 was approximately 2- to 3-fold higher than with [^{123}I]8 through 3 h after injection, consistent with a component of system L transport for [^{76}Br]5 but not for [^{123}I]8. The washout of tracer from the tumor and normal tissues is consistent with a component of reversible amino acid transport such as system L. The kidneys and pancreas had a high initial uptake of [^{76}Br]5 with $47.5 \pm 4.3\%$ ID/g and $27.0 \pm 7.2\%$ ID/g, respectively although this distribution of activity is typical for radiolabeled amino acids.^{13,42,53–56}

Maximal tumor uptake of [^{123}I]8 was observed around the 5 and 30 min time points with values of 2.8 ± 0.8 and $2.7 \pm 0.6\%$ ID/g, respectively. The blood, tumor, and tissue clearance of [^{123}I]8 occurred more rapidly than the brominated analogue [^{76}Br]5 with very little activity remaining at 3 and 24 h after injection of [^{123}I]8. Although activity in the urine and feces was not measured, this result likely reflects differences in renal excretion. For ^{123}I -labeled radiolabeled tracers, rapid metabolism causing deiodination leads to uptake in the thyroid, and our results indicate that significant deiodination of [^{123}I]8 did not occur over the time course of this study. For [^{123}I]8, the highest thyroid uptake occurred at 5 min after injection ($2.5 \pm 0.4\%$ ID/organ) which was lower than the uptake in thyroid observed with [^{76}Br]5 at the same time point ($3.6 \pm 0.7\%$ ID/organ), and the thyroid activity associated with [^{123}I]8 administration decreased to approximately 0.7% ID/organ at the 3 and 24 h time points. Although there is a significantly higher activity in the thyroid at 24 h compared to other tissues, this activity may be due to a small amount of free [^{123}I]iodide in the injected activity and is less than 1% of the administered activity.

In terms of brain tumor imaging, [^{76}Br]5 had slightly higher absolute tumor uptake and higher tumor retention over time compared to [^{123}I]8. Despite the lower tumor uptake, tumor to brain ratios obtained with [^{123}I]8 were higher at all time points studied except at 24 h after injection as depicted in Figure 3. As system L tracers more readily cross the BBB, this result is consistent with the component of system L transport observed in the cell uptake assays using [^{76}Br]5. This resulted in higher normal brain uptake of [^{76}Br]5 compared to [^{123}I]8. Further studies will be needed to determine if the relatively low brain uptake of [^{76}Br]5 is sufficient to visualize nonenhancing brain tumors, which is typical of low grade gliomas but is also seen with some higher grade gliomas.

For the purposes of the biodistribution studies, it is worthwhile to compare the tumor to muscle uptake of both compounds, as they were performed with subcutaneous flank tumors instead of intracranial. The tumor to muscle uptake ratios were generally comparable for [^{76}Br]5 to [^{123}I]8 except at the 24 h time point with a higher tumor to muscle ratio obtained with [^{76}Br]5; see Figure 4.

Small Animal PET/CT Imaging in Mice with Intracranial DBT Gliomas

Four mice with intracranial DBT gliomas were used for PET imaging studies 14 days after implantation. Mice were initially anesthetized with 2% isoflurane and were kept under at 1% isoflurane while undergoing scans. The mice were injected via tail vein with $\sim 55 \mu\text{Ci}$

(~2.04 MBq) of [^{76}Br]**5** and were dynamically imaged from 0 to 60 min, then at 3 and 24 h after injection, static for 15 min. Small animal PET images obtained with [^{76}Br]**5** are presented in Figure 5. For the purposes of clarity and comparison, images seen in the following figures were from the *same* mouse at the different imaging time points.

At 50–60 min after tracer injection, good tumor uptake with relatively low normal brain uptake is observed (right frontal area of the brain); see Figure 5A. Intense activity was present at this time point in the abdomen, related primarily to pancreas, kidney, and urinary excretion. Within 3 h, high uptake is still observed in the major clearance organs (kidneys and bladder) but the amount of activity elsewhere in the body of the mouse has decreased substantially, Figure 5B. The scan also shows an increased uptake and image clarity of the DBT glioma in the brain. At 24 h after injection, further clearance of activity occurred with the most intense residual activity in the DBT brain tumor and urinary bladder. The images are noisy due to the small amount of absolute activity remaining at 24 h, but the uptake in the intracranial DBT tumor is substantially higher than normal tissues, allowing for clear visualization.

SUV tumor uptake values were lower than observed in the biodistribution studies but followed a similar trend with higher values at earlier time points, 0.63 ± 0.06 at 50–60 min (summed) and 0.70 ± 0.13 at 3 h after injection. Tumor to brain ratios had an opposite trend when compared to the observed biodistribution data. Instead, the tumor/brain SUV ratios increased with time from 1.70 ± 0.26 at 50–60 min (summed) to 2.67 ± 0.15 at 3 h, then 3.05 ± 0.30 at 24 h, respectively. This difference in the trend is thought to be due to the difference in location of the tumor.

CONCLUSION

The novel bromovinyl substituted amino acid [^{76}Br]**5** was synthesized in good radiochemical yield and high radiochemical purity from a vinyl tin precursor. In vitro cell uptake assays with mouse DBT glioma cells demonstrated that a mixture of system A and system L transport mediated the majority of uptake of [^{76}Br]**5** while the previously reported iodo analogue, [^{123}I]**8**, was more selective for system A transport. [^{76}Br]**5** demonstrated good uptake and retention in subcutaneous DBT tumors, with maximal tumor uptake around 30 min to an hour postinjection (approximately 3.7–3.8% ID/g). Progressive washout of activity was observed at 3 and 24 h after administration of [^{76}Br]**5** but there was persistent higher tumor uptake compared to most normal tissues at these time points, providing good tumor visualization. Comparative biodistribution studies were performed with the iodo analogue [^{123}I]**8** showing lower overall uptake in normal tissues and less uptake within the tumor, 2.8 ± 0.8 to 2.7 ± 0.6 , at earlier times in the study, 5 and 30 min, respectively.

PET studies using [^{76}Br]**5** in mice implanted with intracranial DBT tumors confirmed the biodistribution results. The tumor is well visualized at 1, 3, and 24 h after injection. At the 24 h time point, high tumor to normal tissue was observed with substantial clearance from normal tissue. This compound represents a lead tracer for mixed system A and system L transport substrates that may provide superior tumor contrast through system A transport while allowing evaluation of the entire brain tumor volume through system L transport

across the BBB. Future work will include evaluation of other analogues of BrVAIB which may have higher retention in tumors at late time points and in other preclinical models of glioma.

EXPERIMENTAL SECTION

Materials and Instrumentation

All chemicals, solvents, and materials were obtained from commercially available sources and used without further purification. Chemicals and solvents were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and VWR (Houston, TX). Thin layer chromatography analyses were carried out on HLF silica gel plates (glass backing), 250 μm /254 nm (Analtech, Newark, DE). Column chromatography was carried out using silica gel, 60 $\text{\AA}$ /40–60 μm , 230–400 mesh, from Sorbent Technologies (Norcross, GA). ^1H and ^{13}C spectra were performed on a Varian 400 MHz spectrometer and were normalized with TMS as 0 ppm. High resolution electrospray mass spectrometry (ESI) was performed by the NIH/NIGMS Biomedical Mass Spectrometry Core Facility at Washington University (St. Louis, MO) on Finnigan LCQ instruments, and values reported were within 5 ppm (H^+ or Na^+). Elemental analyses were performed by Atlantic Microlabs (Norcross, GA) and were within ± 5 ppm of their theoretical values.

Radiochemical high performance liquid chromatography (HPLC) analyses were carried out on an Agilent 1100 series HPLC system. Samples were analyzed with a Phenomenex: Chirex 3126 (D)-penicillamine chiral column using a 3 mM CuSO_4 solution, 15% acetonitrile at a flow rate of 1 mL/min. The production of ammonium [^{76}Br] bromide was performed in the Washington University Cyclotron Facility as previously reported.^{57,58} Sodium (^{123}I) iodide was purchased from MDS Nordion Canada (Ottawa, Ontario, Canada) in a 0.1 M sodium hydroxide solution. C-18 Sep-paks were purchased from Waters, Inc. (Milford, MA). Ion-retardation resin (AG 11A8) was purchased from BioRad (Hercules, CA).

Chemistry

(S)-tert-Butyl 2-((tert-Butoxycarbonyl)amino)-3-hydroxy-2-methylpropanoate (1)—(S)-2-N-(tert-Butoxycarbonyl)-amino-2-methyl-3-hydroxypropanoic acid (1.0 g, 4.6 mmol) was suspended in 25.6 mL of toluene under argon. *N,N*-Dimethylformamide di-*tert*-butyl acetal (5.6 mL, 23 mmol) was added dropwise to the stirring suspension. The solution was heated to 80 $^\circ\text{C}$ and stirred for 2 h, followed by overnight stirring at room temperature. The mixture was washed first with water and then brine. The organic layer was dried (MgSO_4), filtered, and concentrated in vacuo. The crude product was purified via silica gel chromatography, 7:3 hexane/ethyl acetate, and yielded a white crystalline solid (760 mg, 2.76 mmol, 60%). ^1H (CDCl_3): δ 5.32 (s, 1H), 3.98–4.03 (dd, 1H, $J = 11.6, 5.6$ Hz), 3.70–3.75 (dd, 1H, $J = 11.4, 7.6$ Hz), 3.227 (bs, 1H), 1.48 (s, 9H), 1.45 (s, 12H) ppm. ^{13}C (CDCl_3): δ 172.5, 155.2, 81.9, 79.7, 66.7, 60.9, 28.2, 27.7, 20.5 ppm. HRMS (ESI): m/z calculated for $\text{C}_{13}\text{H}_{25}\text{NO}_5 + \text{Na}$ [$\text{M} + \text{Na}$] $^+$, 298.3398; measured, 298.16228. Elemental analysis calculated (%) for $\text{C}_{13}\text{H}_{25}\text{NO}_5$: C 56.71, H 9.15, N 5.09. Found: C 56.84, H 9.25, N 5.11. $[\alpha]_{\text{D}}^{20} +4.7$ (c 0.085, CHCl_3)

(S)-tert-Butyl 2-((tert-butoxycarbonyl)amino)-2-methyl-3-ox-opropanoate (2)—

Dimethyl sulfoxide (0.40 mL, 5.52 mmol) in 3.2 mL of dichloromethane was slowly added to a stirring solution of oxalyl chloride (3.03 mmol, 3.0 mL of a 2.0 M solution) in 0.50 mL of dichloromethane at -78°C . The mixture was stirred for 15 min, and then **1** (760 mg, 2.76 mmol) dissolved into 2.3 mL of dichloromethane was added and stirred for a further hour. Triethylamine (2.0 mL, 14.3 mmol) was added and stirred for an additional 10 min at -78°C . Then the mixture was warmed to room temperature and stirred for a further 30 min. Water was added until the reaction became clear and the layers were separated. The aqueous layer was back extracted with additional dichloromethane. Combined organic fractions were washed once with brine, dried (MgSO_4), filtered, and concentrated. The crude residue was purified via silica gel chromatography, 7:3 hexane/ethyl acetate, to yield the product as a thick, clear oil (629.9 mg, 2.30 mmol, 83.5%). ^1H (CDCl_3): δ 9.52 (s, 1H), 4.46 (bs, 1H), 1.61 (s, 3 H), 1.47 (s, 9H), 1.44 (s, 9H) ppm. ^{13}C (CDCl_3): δ 194.4, 167.7, 154.4, 83.5, 80.2, 66.7, 28.1, 27.6, 18.8 ppm. No HRMS data are reported due to instability of the compound.

(S)-tert-Butyl 4,4-Dibromo-2-((tert-butoxycarbonyl)amino)-2-methylbut-3-

enoate (3)—Zinc dust (90 mg, 1.37 mmol), carbon tetrabromide (457 mg, 1.37 mmol), and triphenylphosphine (362 mg, 1.37 mmol) were added to a flask and suspended into 2.3 mL of dichloromethane. The suspension was stirred for 24 h at room temperature. Compound **2** (188 mg, 0.7 mmol) was dissolved into 0.5 mL of dichloromethane and added to the suspension, and the reaction was allowed to stir for an additional 2 h. The reaction was loaded directly onto a silica gel column and purified (8:2 hexane/ethyl acetate) to yield the purified product as a clear oil (180 mg, 42%). ^1H (CDCl_3): δ 7.00 (s, 1H), 5.76 (s, 1H), 1.64 (s, 3H), 1.49 (s, 9H), 1.45 (s, 9H) ppm. ^{13}C (CDCl_3): δ 170.6, 153.6, 138.8, 82.9 (2 peaks), 28.3, 27.7, 24.9 ppm. HRMS (ESI): m/z calculated for $\text{C}_{14}\text{H}_{23}\text{Br}_2\text{NO}_4 + \text{H} [\text{M} + \text{H}]^+$, 430.1940; measured, 430.0046. $\text{C}_{14}\text{H}_{23}\text{Br}_2\text{NO}_4$: C 39.18, H 5.40, N 3.26, Br 37.24. Found: C 39.46, H 5.45, N 3.27, Br 37.20. $[\alpha]_{\text{D}}^{20} -8.75$ (c 0.08, CHCl_3)

(S)-tert-Butyl (E)-4-Bromo-2-((tert-butoxycarbonyl)amino)-2-methylbut-3-

enoate (4)—Compound **3** (90 mg, 0.26 mmol) was added to a flask with 0.52 mL of dimethylformamide, 0.16 mL of triethylamine and 0.10 mL of dimethyl phosphonate and was stirred at 60°C overnight. The reaction was loaded directly onto a silica gel column and eluted with 9:1 hexane/ethyl acetate to obtain 68 mg (75%). On the basis of the integrations in the ^1H NMR spectrum, it was determined that the isolated product contained a 10:1 mixture of (E)/(Z) isomers. They were readily separated on a silver nitrate doped silica gel column. ^1H (CDCl_3): δ 6.66–6.63 (d, 1H, $J = 8$ Hz - minor isomer), 6.50–6.46, 6.34–6.31 (ABq, 2H, $J = 14$ Hz—major isomer), 6.27–6.25 (d, 1H, $J = 8$ Hz - minor isomer), 5.29 (bs, 1H), 1.57 (s, 3H), 1.47 (s, 9 H), 1.44 (s, 9H) ppm. ^{13}C (CDCl_3): δ 170.7, 153.9, 137.5, 107.4, 82.5, 61.1, 28.3, 28.3, 27.7 ppm. HRMS (ESI): m/z calculated for $\text{C}_{14}\text{H}_{24}\text{BrNO}_4 + \text{Na} [\text{M} + \text{Na}]^+$, 372.2379; measured, 372.0783. $[\alpha]_{\text{D}}^{20} -3.8$ (c 0.105, CHCl_3)

(S)-(E)-2-Amino-4-bromo-2-methylbut-3-enoic Acid (5)—Compound **4** (35 mg, 0.18

mmol) was dissolved in a 1:1 mixture of TFA (0.4 mL) and CH_2Cl_2 (0.4 mL). The solution was stirred for 30 min at room temperature or until the starting material was completely consumed as indicated by TLC (8:2 hexane/ethyl acetate). The reaction mixture was

concentrated down in vacuo and triturated subsequently with Et₂O until a white solid precipitated out. (Quant) ¹H (D₂O): δ 6.70–6.73 (d, *J* = 13.7 Hz, 1H), 6.45–6.49 (d, *J* = 14 Hz, 1H), 1.62 (s, 3H) ppm. HRMS (ESI): *m/z* calculated for C₅H₈BrNO₂ + H [M + H]⁺, 193.9738, measured [M + H]⁺, 193.9811. [*α*]_D²⁰ –4.0 (*c* 0.10, water)

(S)-tert-Butyl (E)-2-((tert-Butoxycarbonyl)amino)-4-iodo-2-methylbut-3-enoate (6)—Chromium chloride (369 mg, 3 mmol) was suspended into 4.3 mL of THF under argon. Compound **2** (138 mg, 0.5 mmol) and iodoform (393 mg, 1 mmol) were dissolved into 2.7 mL of THF and added to the stirring suspension. The mixture was stirred at room temperature overnight. Water was added, and the layers were separated. The aqueous layer was back extracted with ethyl acetate. Combined organic fractions were then washed with brine, dried (MgSO₄), filtered, and concentrated down. The crude residue was purified via silica gel chromatography, 9:1 hexane/ethyl acetate, to yield the product as a yellow oil (97 mg, 0.3 mmol, 55%). ¹H (CDCl₃): δ 6.36–6.39; 6.78–6.81 (qAB, 2H, *J*_{AB} = 14.6 Hz), 5.27 (s, 1H), 1.46 (s, 9H), 1.44 (s, 12H) ppm. ¹³C (CDCl₃): δ 170.5, 153.9, 145.1, 82.4, 79.9, 77.3, 62.8, 28.3, 27.7, 27.6 ppm. HRMS (ESI): *m/z* calculated for C₁₄H₂₄INO₄ + H [M + H]⁺, 398.0750; measured, 398.3121. [*α*]_D²⁰ –8.0 (*c* 0.10, CHCl₃)

(S)-tert-Butyl (E)-2-((tert-Butoxycarbonyl)amino)-2-methyl-4-(trimethylstannyl)but-3-enoate (7)—Compound **6** (38.2 mg, 0.11 mmol) was dissolved into 2.2 mL of THF and degassed with argon for 15 min. Hexamethyditin (71 mg, 0.2 mmol) and tetrakis(triphenylphosphine)palladium(0) (13 mg, 0.011 mmol) were added, and the solution was degassed for a further 10 min. The mixture was heated to 50 °C and stirred for 2 h. Once cooled to room temperature, the reaction solvent was concentrated under reduced pressure and purified via silica gel chromatography (7:1:0.1% hexane/ethyl acetate/trimethylamine) to yield a thick, pale yellow oil (32 mg, 0.08 mmol, 75%). ¹H (CDCl₃): δ 5.89–5.95, 6.12–6.17 (qAB, 2H, *J*_{AB} = 19.0 Hz), 5.13 (s, 1H), 1.3 (s, 21H), 0.00 (s + TMS, 9H) ppm. ¹³C (CDCl₃): δ 171.7, 154.2, 146.5, 128.9, 81.3, 62.3, 28.3, 27.7, –9.7 (3 C) ppm. HRMS (ESI): *m/z* calculated for C₁₇H₃₃NO₄Sn + Na [M + Na]⁺, 458.1330; measured, 458.1320. [*α*]_D²⁰ –6.3 (*c* 0.095, CHCl₃).

(S)-2-Amino-2-methyl-4-iodo-3-(E)-butenoic Acid (8)—Compound **6** (40 mg, 0.17 mmol) was dissolved into an 1:1 mixture of TFA (0.5 mL) and CH₂Cl₂ (0.5 mL). The mixture was stirred for 30 min at room temperature or until the starting material was completely consumed as indicated by TLC (8:2 hexane/ethyl acetate). The reaction mixture was concentrated in vacuo and triturated subsequently with Et₂O until a white solid precipitated out. (Quant) NMR spectra agreed with previously published literature.⁴²

Radiosynthesis of [⁷⁶Br]5

[⁷⁶Br] was produced using the CS15 cyclotron in the Washington University Cyclotron Facility using the ⁷⁶Se(p, n)⁷⁶Br nuclear reaction on a ⁷⁶Se-enriched Cu₂Se target.^{57,58} Bromine-76 was purified via the dry distillation method presented by Tang et. al⁵⁸ and collected in Milli-Q water. The purified isotope was used as was received.

Incorporation of ^{76}Br was achieved in a mixture of the starting vinyltin compound (80 μg in 25 μL of ethanol) and peracetic acid (100 μL of a 3% solution in acetic acid) at room temperature in 15 min. Efficiency of the radiolabeling was determined using radiometric instant thin layer chromatography (radio-iTLC) on glass microfiber TLC paper impregnated with silica gel. The radio-iTLC was developed using an 8:2 mixture of hexane and ethyl acetate. The compound was then deprotected in 300 μL of a 4 N HCl solution heated in an oil bath to 110 $^{\circ}\text{C}$ for 30 min. The reaction was loaded directly onto an ion-retardation column and eluted with water. The pH of the compound was always 7.0 after elution from the ion retardation column. It was then loaded and passed through a C18-sep pak. Further collection was accomplished with either water or saline.

The radiochemical purity and specific activity were assessed using a chiral analytical HPLC column. Specific activities were calculated based on HPLC calibration curves generated with nonradiolabeled standards.

Radiosynthesis of [^{123}I]8

Sodium [^{123}I] iodide was purchased in a 0.1 M sodium hydroxide (NaOH) solution from MDS Nordion Canada. Incorporation of ^{123}I was achieved in a mixture of the starting vinyltin compound (80 μg in 25 μL ethanol), hydrogen peroxide (100 μL of a 3% solution in ethanol), and an equal part of 0.1 M HCl at room temperature in 15 min. Efficiency of the radiolabeling was estimated using radio-iTLC as described previously.

The reaction was quenched with sodium metabisulfite and sodium bicarbonate, and the organic layer was pipetted out. The resulting aqueous layer was extracted twice with dichloromethane. Combined organic fractions were dried down with gentle heat and nitrogen flow. The compound was deprotected by treatment with 300 μL of a 4 N HCl solution heated in an oil bath to 110 $^{\circ}\text{C}$ for 30–60 min. The reaction was loaded directly onto an ion-retardation column and eluted with water. If the pH of the compound was between 6.0 and 7.0, the reaction was loaded onto a C18-sep pak and isolated with water or saline. In rare cases, if the pH of the compound was above or below the desired range, it was loaded onto another ion-retardation column and collected again. The radiochemical purity and specific activity were assessed using a chiral analytical HPLC column and iTLC.

Cell Uptake Assays

Cell uptake assays were performed using the cluster tray method as reported in the literature^{59,60} to evaluate the influx of the radiotracers and to minimize efflux that can occur over time for some amino acid transporters. Mouse DBT gliomas cells were cultured at 1×10^5 cells/well in 24-well plates (Corning, NY, USA) for 48 h in a 5% CO_2 atmosphere in 10% FBS DMEM culture medium. Multiple inhibitors were used, both with and without the addition of sodium. The phosphate-buffered saline solution contained final concentrations of 105 mM sodium chloride (NaCl), 3.8 mM potassium chloride (KCl), 1.2 mM potassium bicarbonate (KHCO_3), 25 mM sodium phosphate dibasic (Na_2HPO_4), 0.5 mM calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 1.2 mM magnesium sulfate (MgSO_4), and 5.6 mM D-glucose. The sodium-free phosphate-buffered choline solution was prepared with identical salt concentrations to the phosphate-buffered saline solution except choline chloride and

choline phosphate (dibasic) salts were substituted for sodium chloride and sodium phosphate (dibasic), respectively.

The control medium contained 10 mM of sucrose to maintain consistent osmolality. The following inhibitors were used for the cell uptake assays: *N*-methyl- α -aminoisobutyric acid (MeAIB, 10 mM, system A), (*R,S*)-(*endo,exo*)-2-aminobicyclo(2,2,1)heptane-2-carboxylic acid (BCH, 10 mM, system L), and a mixture of *L*-alanine, *L*-serine, and *L*-cysteine (ASC, 3.3 mM of each amino acid, small neutral amino acid transport systems).

The assays were performed as described previously^{60,61} at pH 7.40 with each condition performed in triplicate (per plate) using two plates. Briefly, cells were washed twice with 37 °C assay buffer (2 mL) prior to initiating the assay. For each tracer, [⁷⁶Br]**5** or [¹²³I]**8**, solutions containing approximately 1.0 mCi (0.037 GBq)/mL were prepared in the appropriate assay buffer, and then 20 μ L of the radioactive tracer was added to appropriate buffer with or without inhibitors. Cells were incubated with radiotracers in the designated assay buffer (0.4 mL total volume) for 60 s at 37 °C. The assay buffer was then discarded from each well followed by washes (3 $\times$ 1 mL) with ice-cold buffer to remove extracellular radiotracer. The cells were lysed using 0.3 mL of 0.2% SDS/0.2 M sodium hydroxide at room temperature for 30 min. A 150 μ L portion of the lysate from each well was collected and assessed for radioactivity using a γ counter (1 min/tube) to determine the amount of radioactivity taken up by the cells (given as counts per minute). Protein content was determined using 3 $\times$ 30 μ L portions using the bicinchoninic acid method (Pierce, BCA protein assay kit). Standard solutions of each assay condition with radioactivity were also counted to determine the average amount of activity added to each well of the assay. The amount of radioactivity per well was normalized based on the amount of radioactivity added and the protein content of each cell. The uptake data were expressed as percent of uptake relative to control, and each plate was analyzed with Microsoft Excel.

DBT Tumor Model

All animal studies were conducted according to protocols approved by the Animal Studies Committee at Washington University School of Medicine. For the intracranial tumors, DBT tumor cells (1×10^4 suspended in a volume of 8 μ L) were implanted in the right mid-cerebrum of male BALB/c mice (21–24 g) as described previously.^{62,63} For subcutaneous tumors, the DBT cells (5×10^5 cells suspended in a volume of 50 μ L) were injected subcutaneously into the flanks of male BALB/c mice (23–30 g).⁶³ Tumor bearing mouse models were used 14 days after implantation.

Biodistribution Studies with [⁷⁶Br]**5** and [¹²³I]**8** in Mice with Subcutaneous DBT Tumors

Biodistribution studies were carried out with male BALB/c mice ($n = 20$ /compound studied) implanted with subcutaneous flank DBT glioma tumor cells, at 14 days after implantation. Mice were anesthetized with 1% isoflurane–oxygen and were intravenously injected (tail vein) with the radiolabeled compound. The mice were euthanized at various time points ranging from 5 min to 24 h ($n = 4$ at each time point). Tumors, organs, and tissues of interest were harvested, weighed, and radioactivity was measured in γ -counter. Data were calculated as the percent injected dose (% ID) per gram tissue.

Small Animal PET/CT Imaging in Mice with Intracranial DBT Gliomas

Small animal PET/CT imaging was carried out in four mice implanted with intracranial DBT gliomas at 14 days after implantation. PET imaging studies were performed on the same day, in parallel with biodistribution studies. Mice were anesthetized with 1% isoflurane–oxygen, intravenously injected (tail vein) with the radiolabeled compound and kept under a steady stream of 1% isoflurane–oxygen during each imaging time point. Images were collected from 0 to 60 min (dynamic) and then at 3 and 24 h after injection (static), for 15 min. The mice were euthanized after the completion of PET imaging studies. The brains were fixed in a 4% paraformaldehyde solution for 2 weeks and then subjected to histologic analysis using hematoxyline and eosin staining to verify the presence and location of the brain tumor (data not shown).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

% ID/g	percent injected dose per gram of tissue
BCH	(<i>R,S</i>)-(endo,exo)-2-aminobicyclo(2,2,1)heptane-2-carboxylic acid
BrVAIB	(<i>S</i>)-2-amino-2-methyl-4-bromo-3-(<i>E</i>)-butenoic acid
CBr ₄	carbon tetrabromide
CH ₃ CO ₃ H	peroxyacetic acid
(COCl) ₂	oxalyl chloride
DBT	delayed brain tumor
dc	decay corrected
Et ₂ O	diethyl ether
[^{18}F]FDG	[^{18}F]-fluorodeoxyglucose
IVAIB	(<i>S</i>)-2-amino-2-methyl-4-iodo-3-(<i>E</i>)-butenoic acid
NH ₄ [^{76}Br]	ammonium [^{76}Br]bromide

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^aReagents and conditions: (a) $(\text{CH}_3)_2\text{NCH}[\text{OC}(\text{CH}_3)_3]_2$, toluene, 80 °C, 2 h, rt overnight; (b) (i) DMSO, $(\text{COCl})_2$, DCM, -78 °C, 15 min, (ii) **1**, DCM, -78 °C 1 h, (iii) NEt_3 , -78 °C to rt 30 min; (c) Zn dust, CBr_4 , PPh_3 , rt, 24 h, then **2**, rt, 2 h; (d) $\text{HOP}(\text{O})(\text{OMe})_2$, DMF, NEt_3 , 60 °C overnight; (e) TFA, Et_2O , rt, 30 min.

Scheme 1.

Multistep Synthesis of the Nonradioactive, HPLC Standard BrVAIB (**5**)^a

^aReagents and conditions: (a) CrCl_2 , CHI_3 , THF, rt, overnight; (b) $[(\text{CH}_3)_3\text{Sn}]_2$, $\text{Pd}^0(\text{PPh}_3)_4$, THF, 55 °C, 2 h ; (c) TFA, Et_2O , rt, 30 min.

Scheme 2.

Synthesis of the Nonradioactive IVAIB (8) and the Labeling Precursor (7)^a

^aReagents and conditions: (a) CH₃CO₃H, AcOH, NH₄[⁷⁶Br], H₂O, rt, 20 min; (b) HCl, 110 °C, 30 min.

Scheme 3.
Radiosynthesis of [⁷⁶Br]BrVAIB^a

Figure 1.

Analytical HPLC analysis of $[^{76}\text{Br}]\mathbf{5}$ seen with radiometric detection (not co-injected). Free ^{76}Br has a retention time of 3.2 min, with a corresponding peak in UV (also solvent front). $[^{76}\text{Br}]\mathbf{5}$ has a retention time of 4.4 min. The corresponding UV peak at 4.2 min is thought to be deprotected residual starting material.

Figure 2.

In vitro cell uptake assays of [⁷⁶Br]5 and [¹²³I]8 in DBT glioma cells in the presence and absence of sodium and known amino acid transport inhibitors. The data presented in the figure are normalized to the amount of protein per well in addition to the amount of activity added per sample well. Each bar presented above is an average of eight replicates per condition, and the error bars are indicative of standard deviation (SD): (***) $p = 0.0002$; (**) $p = 0.0013$.

Figure 3. Average tumor/brain uptake ratios of $[^{76}\text{Br}]5$ and $[^{123}\text{I}]8$ over time. The error bars indicate standard deviation: (*) $p = 0.005$.

Figure 4.

Average tumor/muscle uptake ratios of $[^{76}\text{Br}]5$ and $[^{123}\text{I}]8$ over time. The error bars indicate standard deviation: (*) $p = 0.007$.

Figure 5.

(A) Axial view (i) of the mouse brain and coronal slice (ii) at 50–60 min (summed) after injection of $[^{76}\text{Br}]\mathbf{5}$. A maximal intensity projection (MIP) of 0–60 min (summed) can be seen in (iii). Localization of compound in the brain tumor is observed at this time, with high uptake in the excretory organs. T = tumor. (B) Axial view of the mouse brain (i), sagittal view (ii), a MIP of PET and CT combined (iii) of the whole mouse and a MIP of PET only (iv) at 3 h after injection of $[^{76}\text{Br}]\mathbf{5}$. T = tumor, B = bladder, K = kidney(s). (C) Axial view (i), sagittal view (ii), a MIP (iii) of PET and CT combined and a MIP of PET only at 24 h after injection. T = tumor, B = bladder.

Table 1

Summary of Transport of [^{76}Br]5 and [^{123}I]5 by System A, System L, and Other Neutral Amino Acid Transporters^a

system	types of compounds transported	sodium dependency	mechanism of transport	known system inhibitors	[^{76}Br]5 system transport	[^{123}I]8 system transport
A	small, neutral amino acids (Ala, Gly, etc.), recognizes N-methylated derivatives	yes	co-transport with Na^+ , unidirectional	MeAIB	yes	yes
L	large, branched, neutral amino acids (Leu, Phe, Tyr, etc.)	no	exchange (LAT1, LAT2), facilitated diffusion (LAT3, LAT4)	BCH	yes	no
neutral amino acid transport (including ASC)	small, neutral amino acids (Ala, Ser, Cys, etc.)	yes	multiple mechanisms	Ala, Cys, Ser	yes	no

^a Amino acid transporter system transport is designated as yes or no based on the results of the in vitro cell uptake assays.

Table 2 Whole Body Biodistribution (% ID/g) of [^{76}Br]5 in BALB/c Mice with Subcutaneous Flank DBT Tumors

organ or tissue	5 min	30 min	1 h	3 h	24 h
blood	5.75 $\pm$ 0.44	1.66 $\pm$ 0.16	1.22 $\pm$ 0.19	0.87 $\pm$ 0.44	0.37 $\pm$ 0.04
lung	6.20 $\pm$ 0.41	1.73 $\pm$ 0.16	1.15 $\pm$ 0.22	0.54 $\pm$ 0.07	0.27 $\pm$ 0.05
liver (all)	4.57 $\pm$ 0.27	1.63 $\pm$ 0.33	1.03 $\pm$ 0.48	0.34 $\pm$ 0.09	0.1 $\pm$ 0.02
spleen	8.20 $\pm$ 0.71	4.05 $\pm$ 0.55	2.30 $\pm$ 0.82	0.59 $\pm$ 0.21	0.18 $\pm$ 0.03
kidney	47.50 $\pm$ 4.26	8.95 $\pm$ 0.91	5.49 $\pm$ 0.98	1.29 $\pm$ 0.43	0.23 $\pm$ 0.02
muscle	1.54 $\pm$ 0.18	1.75 $\pm$ 0.77	0.98 $\pm$ 0.12	0.4 $\pm$ 0.20	0.08 $\pm$ 0.01
fat	1.36 $\pm$ 0.38	0.85 $\pm$ 0.71	0.31 $\pm$ 0.20	0.17 $\pm$ 0.04	0.04 $\pm$ 0.01
heart	2.92 $\pm$ 0.29	2.21 $\pm$ 0.18	1.67 $\pm$ 0.27	0.67 $\pm$ 0.15	0.13 $\pm$ 0.02
brain	0.54 $\pm$ 0.04	0.42 $\pm$ 0.02	0.38 $\pm$ 0.04	0.31 $\pm$ 0.06	0.09 $\pm$ 0.01
bone	2.13 $\pm$ 0.43	1.95 $\pm$ 0.29	1.18 $\pm$ 0.64	0.46 $\pm$ 0.20	0.1 $\pm$ 0.04
thyroid	3.60 $\pm$ 0.69	1.63 $\pm$ 0.12	1.26 $\pm$ 0.18	0.53 $\pm$ 0.08	0.17 $\pm$ 0.06
pancreas	27.04 $\pm$ 7.15	24.35 $\pm$ 4.06	14.31 $\pm$ 8.12	2.48 $\pm$ 1.17	0.17 $\pm$ 0.02
tumor	2.52 $\pm$ 0.64	3.79 $\pm$ 0.95	3.72 $\pm$ 0.43	1.65 $\pm$ 0.40	0.25 $\pm$ 0.03

Table 3 Whole Body Biodistribution (% ID/g) of [^{123}I]8 in BALB/c Mice with Subcutaneous Flank DBT Tumors

organ or tissue	5 min	30 min	1 h	3 h	24 h
blood	3.86 ± 0.50	1.07 ± 0.16	0.37 ± 0.05	0.07 ± 0.02	0.0005 ± 0.0000
lung	3.54 ± 0.31	1.01 ± 0.28	0.33 ± 0.06	0.06 ± 0.02	0.0007 ± 0.0006
liver (all)	2.83 ± 0.25	0.91 ± 0.20	0.25 ± 0.03	0.07 ± 0.02	0.0006 ± 0.0002
spleen	3.92 ± 0.99	2.23 ± 0.34	0.68 ± 0.12	0.15 ± 0.05	0.0012 ± 0.0010
kidney	31.27 ± 6.91	9.90 ± 3.24	2.68 ± 0.55	0.39 ± 0.09	0.0032 ± 0.0017
muscle	1.65 ± 0.05	1.41 ± 0.22	0.68 ± 0.17	0.12 ± 0.06	0.0011 ± 0.0006
fat	0.87 ± 0.26	0.22 ± 0.09	0.19 ± 0.07	0.04 ± 0.01	0.0000 ± 0.0000
heart	2.30 ± 0.02	1.78 ± 0.35	0.90 ± 0.06	0.18 ± 0.05	0.0005 ± 0.0005
brain	0.27 ± 0.03	0.17 ± 0.04	0.27 ± 0.35	0.07 ± 0.00	0.0010 ± 0.0003
bone	1.94 ± 0.28	1.20 ± 0.21	0.55 ± 0.39	0.39 ± 0.35	0.0042 ± 0.0020
thyroid	2.51 ± 0.39	1.38 ± 0.38	0.97 ± 0.28	0.65 ± 0.29	0.6527 ± 0.2135
pancreas	29.26 ± 8.15	21.03 ± 4.38	5.75 ± 1.20	0.99 ± 0.46	0.0052 ± 0.0021
tumor	2.78 ± 0.83	2.68 ± 0.56	1.53 ± 0.66	0.65 ± 0.16	0.0012 ± 0.0007