Brief Articles

Facile Stereospecific Synthesis and Biological Evaluation of (*S*)- and (*R*)-2-Amino-2-methyl-4-[^{123}I]iodo-3-(*E*)-butenoic Acid for Brain Tumor Imaging with Single Photon Emission Computerized Tomography

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Both enantiomers of 2-amino-2-methyl-4-iodo-3-(*E*)-butenoic acid (IVAIB, **5**) were radioiodoinated in 65−72% yield. (*S*)-IVAIB entered 9L gliosarcoma cells primarily via A-type transport in vitro with higher uptake than (*R*)-IVAIB. Biodistribution studies in rats with 9L gliosarcoma brain tumors demonstrated higher tumor to brain ratios with (*S*)-IVAIB (75:1 at 1 h) than (*R*)-IVAIB (7.7:1). In this model, (*S*)-IVAIB is superior to (*R*)-IVAIB and is a promising radiotracer for brain tumor imaging.

Introduction

A variety of radiolabeled amino acids have been developed as potential tumor imaging agents for positron emission tomography (PET) and single photon emission computerized tomography (SPECT).[1] Many radiolabeled amino acids developed for tumor imaging enter cells via the A-type (sodium dependent) and/or the L-type (sodium independent) transport systems, which are upregulated in many neoplasms.[1] The L-type substrate [^{123}I]IMT represents the most widely used amino acid for tumor imaging with SPECT.[1,2] Currently, no iodinated amino acids that are selective A-type substrates have been described.

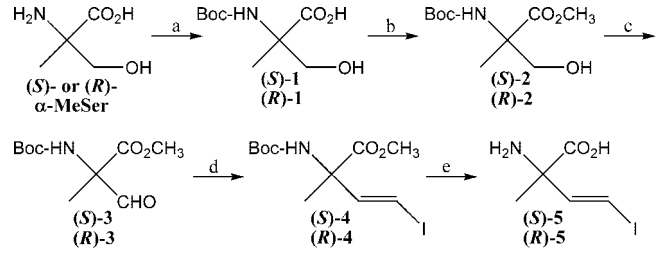
In this study, we describe the synthesis and biological evaluation of (*S*)- and (*R*)-2-amino-2-methyl-4-iodo-3-(*E*)-butenoic acid (IVAIB, **5**), which are iodinated analogues of the A-type transport substrate AIB. (*S*)-**5** and (*R*)-**5** were labeled with iodine-123 or iodine-131 for cell uptake assays and biodistribution studies in rats with intracranial 9L gliosarcoma tumors to evaluate their potential for tumor imaging.

Results and Discussion

Chemistry and Radiolabeling. (*S*)-**5** and (*R*)-**5** were prepared separately from commercially available (*S*)- and (*R*)-α-methylserine as shown in Scheme 1. In the key synthetic step, reaction of **3** with iodoform in tetrahydrofuran using chromium(II) chloride as a catalyst[3] gave exclusively *trans*-(*E*)-isomers of (*S*)-**4** and (*R*)-**4** as determined by ^{1}H NMR. No attempt was made to prepare the corresponding *Z* isomers. The stannyl labeling precursors (*S*)-**6** and (*R*)-**6** were prepared from (*S*)-**4** and (*R*)-**4** using hexamethylditin and catalytic tetrakis-(triphenylphosphine)palladium(0) as shown in Scheme 2.[4]

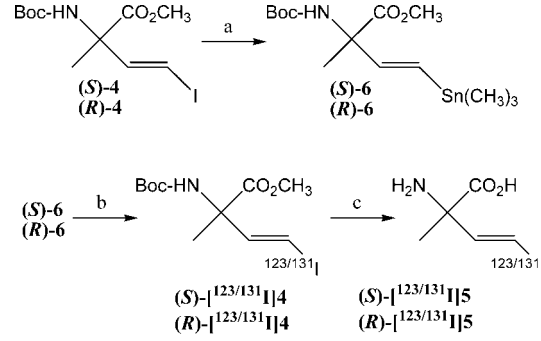
Radioiodinated (*S*)-**5** and (*R*)-**5** were prepared using no-carrier-added (NCA) sodium [^{123}I]iodide or sodium [^{131}I]iodide under oxidizing conditions followed by deprotection with aqueous hydrochloric acid (Scheme 2). The radioiodinated products were purified by ion-retardation resin chromatography.

Scheme 1. Synthesis of Nonradioactive Amino Acids (*S*)- and (*R*)-IVAIB (**5**)[a]



[a] Reagents and conditions: (a) $(Boc)_2O$, room temp; (b) $(CH_3)_3SiCHN_2$, MeOH/benzene, room temp; (c) DMSO, $(COCl)_2$, CH_2Cl_2, −50 to −60 °C, then Et_3N; (d) CHI_3, $CrCl_2$, THF, room temp; (e) HCl, 120 °C.

Scheme 2. Preparation of Labeling Precursor **6** and Radiosynthesis of (*S*)- and (*R*)-[$^{123/131}$I]IVAIB (**5**)[a]



[a] Reagents and conditions: (a) $(CH_3)_3Sn-Sn(CH_3)_3$, $Pd^0(PPh_3)_4$, THF, 50 °C; (b) [$^{123/131}$I]NaI, H_2O_2, HCl; (c) HCl, 110 °C.

The procedure required approximately 100 min with decay-corrected yields (dcy) of 72 ± 18% (n = 10, (*S*)-[$^{123/131}$I]IVAIB) and 65 ± 16% (n = 9, (*R*)-[$^{123/131}$I]IVAIB) in over 99% radiochemical purity as measured by radiometric TLC. On the basis of 200 nmol of tin precursor utilized for labeling and approximate yields of 1 mCi of product, the minimum specific activities for (*S*)- and (*R*)-[$^{123/131}$I]**5** are estimated at 5 mCi/μmol. The final product was not assayed for the presence of tin.

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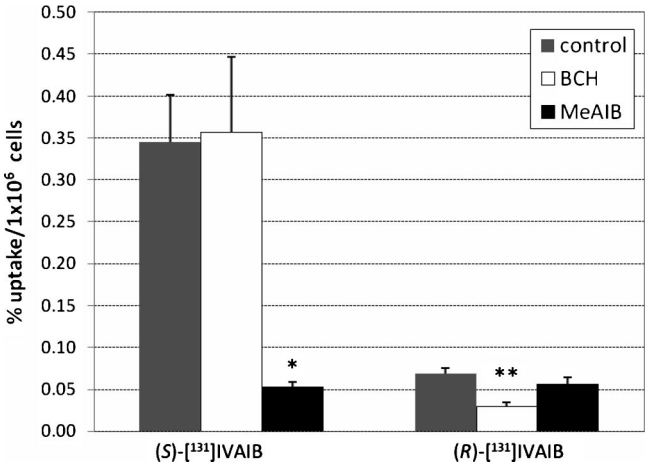

Figure 1. 9L gliosarcoma cell uptake at 30 min of (*S*)- and (*R*)-[^{131}I]IVAIB (**5**) with or without inhibitors expressed as percent uptake of the initial dose per 1 million cells (% uptake/1 × 10^6 cells). Uptake was determined after 30 min incubations and normalized for dose and number of cells. *p* values represent comparisons of uptake in the presence of inhibitor to control uptake for each radiotracer (one-way ANOVA): (∗) 85% reduction vs contral, $p < 0.01$; (∗∗) 57% reduction vs control, $p < 0.001$. Error bars indicate standard deviation.

Cell Uptake Assays. Amino acid uptake inhibition assays were performed with (*S*)-[^{131}I]**5** and (*R*)-[^{131}I]**5** using cultured 9L gliosarcoma cells in the presence and absence of the amino acid transport inhibitors *N*-methyl-α-aminoisobutyric acid (*N*-MeAIB, A-type transport inhibitor) and 2-aminobicyclo[2.2.1]-heptane-2-carboxylic acid (BCH, L-type transport inhibitor).[5–8] The results of these amino acid uptake assays are depicted in Figure 1.

In the absence of inhibitors, (*S*)-[^{131}I]**5** showed a significantly higher level of intracellular accumulation (0.35 ± 0.07% of the initial dose per million cells) in 9L gliosarcoma cells compared to (*R*)-[^{131}I]**5** (0.07 ± 0.01%, $p < 0.001$). In the case of (*S*)-[^{131}I]**5**, no significant uptake inhibition occurred with BCH, whereas *N*-MeAIB blocked 85% of uptake relative to controls ($p < 0.01$). In contrast, uptake of (*R*)-[^{131}I]**5** was significantly inhibited by BCH (57% versus control, $p < 0.001$) while no significant inhibition occurred with *N*-MeAIB. These results demonstrate that (*S*)-**5** is a selective substrate for A-type transport in 9L gliosarcoma cells in vitro and that the absolute configuration at the α-carbon affects the amount and selectivity of cellular transport.

In Vivo Biodistribution Studies in Tumor Bearing Rats. The tissue distribution of (*S*)-[^{123}I]**5** and (*R*)-[^{131}I]**5** in Fischer rats with intracranial 9L tumors is presented in Table 1. The tumor uptake of radioactivity with (*S*)-[^{123}I]**5** ranged from 4.70% to 6.84% injected dose per gram tissue (%ID/g) and was significantly higher than in normal brain tissue ($p < 0.001$ at all time points). The tumor to normal brain uptake ratios ranged from 71:1 to 81:1. The low brain uptake of (*S*)-[^{123}I]**5** in vivo is consistent with the preferential A-type transport observed in the cell uptake assays because A-type transport substrates including [^{11}C]MeAIB do not cross the intact blood−brain barrier,[9,10] and similar high tumor to normal brain ratios were obtained with fluorinated analogues of AIB, [^{18}F]FAMP, and [^{18}F]*N*-MeFAMP, which are also A-type transport substrates.[11,12]

With (*R*)-[^{131}I]**5**, tumor uptake ranged from 0.19 to 0.51 %ID/g with tumor to normal brain ratios ranging from 7.6:1 to 10:1. Unlike (*S*)-[^{123}I]**5**, the amount of tumor uptake observed with (*R*)-[^{131}I]**5** was not significantly different from blood levels (%ID/g) at any of the time points evaluated. These results demonstrate that the tumor uptake of (*S*)-[^{123}I]**5** is not due to an abnormal blood−brain barrier in the tumor tissue because the (*S*)- and (*R*)-enantiomers of IVAIB have identical physiochemical properties but very different uptake profiles. The tumor uptake of (*S*)-[^{123}I]**5** was significantly higher than (*R*)-[^{131}I]**5** at all time points examined ($p < 0.001$ at each time point) with much higher tumor to brain ratios. As in the cell uptake studies, these findings demonstrate that the absolute configuration of **5** is a key determinant of biological behavior.

In normal tissues, (*S*)-[^{123}I]**5** demonstrated the highest uptake of radioactivity in the pancreas (10.7 %ID/g) and kidneys (22.9 %ID/g) at 60 min, similar to other studies with A-type and L-type amino acid radiotracers.[10–16] The remainder of the measured tissues had relatively low uptake, suggesting that (*S*)-**5** may also be suitable for imaging tumors outside the central nervous system. As with the (*S*)-enantiomer, the highest uptake with (*R*)-[^{131}I]**5** occurred in the pancreas and kidneys and the lowest uptake occurred in the brain. The low thyroid uptake with both radiotracers indicates that free iodide was not generated during the time course of the study.

Conclusions

Both enantiomers of IVAIB can be labeled with I-123 and I-131 in high radiochemical yield (>65% dcy) and high radiochemical purity. The enantiomers of IVAIB have markedly different biological properties. (*S*)-IVAIB is primarily an A-type amino acid transport substrate in cell uptake assays, while (*R*)-IVAIB is a relatively poor amino acid transport substrate and undergoes little if any A-type transport. (*S*)-[^{123}I]IVAIB showed rapid uptake and persistent retention of radioactivity in rodent 9L gliosarcoma brain tumors with excellent tumor to brain ratios ranging from 71:1 to 81:1, consistent with its in vitro mechanism of transport. In contrast, (*R*)-[^{131}I]IVAIB demonstrated significantly lower tumor uptake and tumor to brain ratios (7.6:1 to 10:1). On the basis of these results, (*S*)-IVAIB is a promising SPECT agent for brain tumor imaging.

Experimental Section

Materials and Instrumentation. All chemicals, solvents, and materials used were obtained from commercially available sources and used without purification. Chemicals were purchased from Aldrich Chemicals Co. (Milwaulkee, WI) and Sigma Chemical Co. (St. Louis, MO), and solvents were purchased from Aldrich Chemicals and VWR Scientific Products (West Chester, PA). 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) and are uncorrected. ^{1}H NMR spectra were recorded on Varian 400 or 300 MHz spectrometers, 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) and were within ±0.4% of the theoretical values. Mass spectra were done on a JEOL JMS-SX102/SX102A/E or VG 70-S double focusing mass spectrometer using high-resolution electrospray ionization (ESI).

Sodium [^{123}I]iodide was purchased from MDS Nordion (Ottawa, Ontario, Canada), and sodium [^{131}I]iodide was purchased from PerkinElmer (Shelton, CT), both in 0.1 N sodium hydroxide solution. Alumina N and C-18 SepPaks were purchased from Waters, Inc. (Milford, MA). Ion-retardation resin (AG 11A8 50–100 mesh) was purchased from BioRad (Hercules, CA).

Thin-layer chromatograms of the radiolabeled compounds were analyzed using silica TLC plates. All animal experiments were

Table 1. Biodistribution as Percent of Injected Dose per Gram (%ID/g) of Radioactivity in Tissues of 9L Gliosarcoma Tumor-Bearing Rats after Intravenous Administration of (*S*)-[^{123}I]IVAIB and (*R*)-[^{131}I]IVAIB

	(*S*)-[^{123}I]IVAIB			(*R*)-[^{131}I]IVAIB		
tissue	30 min	60 min	120 min	30 min	60 min	120 min
blood	1.33 ± 0.13	1.22 ± 0.23	1.03 ± 0.14	0.22 ± 0.04	0.69 ± 0.09	0.56 ± 0.05
heart	1.31 ± 0.13	1.40 ± 0.04	1.20 ± 0.20	0.19 ± 0.04	0.43 ± 0.07	0.34 ± 0.04
lung	1.24 ± 0.16	1.41 ± 0.17	0.98 ± 0.07	0.27 ± 0.07	0.65 ± 0.10	0.45 ± 0.05
liver	1.62 ± 0.15	1.62 ± 0.14	1.27 ± 0.09	0.31 ± 0.06	0.60 ± 0.08	0.37 ± 0.06
pancreas	11.9 ± 1.7	10.7 ± 1.6	7.7 ± 1.1	1.21 ± 0.26	1.86 ± 0.04	0.78 ± 0.22
spleen	1.54 ± 0.09	1.73 ± 0.37	1.08 ± 0.11	0.30 ± 0.040	0.50 ± 0.08	0.31 ± 0.03
kidney	24.0 ± 3.1	22.9 ± 2.58	18.4 ± 1.5	2.91 ± 0.45	3.43 ± 0.72	1.76 ± 0.18
muscle	1.35 ± 0.10	1.70 ± 0.18	1.26 ± 0.21	0.26 ± 0.03	0.40 ± 0.05	0.26 ± 0.03
brain	0.058 ± 0.014	0.091 ± 0.043	0.096 ± 0.025	0.018 ± 0.009	0.066 ± 0.012	0.051 ± 0.01
tumor	4.70 ± 1.05	6.81 ± 1.24	6.84 ± 1.36	0.19 ± 0.08	0.51 ± 0.10	0.39 ± 0.06
bone	0.51 ± 0.13	0.74 ± 0.26	0.64 ± 0.08	0.13 ± 0.02	0.34 ± 0.04	0.26 ± 0.05
testis	0.49 ± 0.04	0.58 ± 0.04	0.64 ± 0.10	0.09 ± 0.01	0.26 ± 0.04	0.23 ± 0.03
thyroid per organ	0.16 ± 0.04	0.24 ± 0.019	0.18 ± 0.01	0.08 ± 0.01	0.25 ± 0.04	0.36 ± 0.04
tumor/brain ratio	81	75	71	10	7.7	7.6

carried out under humane conditions and were approved by the Institutional Animal Use and Care Committee (IUCAC) and Radiation Safety Committee at Emory University.

Chemistry. (*S*)- and (*R*)-2-*N*-(*tert*-Butoxycarbonyl)amino-2-methyl-3-hydroxy-propanoic Acid (1). (*S*)-(+)-2-Amino-2-methyl-3-hydroxypropanoic acid or (*R*)-(−)-2-amino-2-methyl-3-hydroxypropanoic acid (α-methylserine) (268 mg, 2.25 mmol, Acros Organics, Morris Plains, NJ) was suspended in 7 mL of methanol−triethylamine (9:1 v/v), and 1 N sodium hydroxide (∼2 mL) was added with stirring until all solid was dissolved, at which point di-*tert*-butyl dicarbonate (966 mg, 4.43 mmol) was added in one portion. The mixture was stirred at room temperature overnight. The organic solvent was removed under reduced pressure, and 10 mL of ethyl acetate was added. The pH of the aqueous phase was adjusted to 2 with 3 N hydrochloric acid, and the organic phase was separated. The aqueous phase was saturated with sodium chloride and extracted with ethyl acetate (3 × 10 mL). The combined organic phases were dried over magnesium sulfate, filtered, and concentrated to dryness under reduced pressure. The products were white solids that were used without further purification. (*S*)-2-*N*-(*tert*-butoxycarbonyl)amino-2-methyl-3-hydroxypropanoic acid ((*S*)-**1**): 480 mg (97%); ^{1}H NMR ($CDCl_3$) δ 1.47 (9H, s), 1.52 (3H, s), 3.79, 3.82 (1H, d, J = 12 Hz), 3.93, 3.96 (1H, d, J = 12 Hz), 5.51 (1H, broad s); mp 114–117 °C (dec). (*R*)-2-*N*-(*tert*-butoxycarbonyl)amino-2-methyl-3-hydroxypropanoic acid ((*R*)-**1**): 445 mg (90%); ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s), 1.51 (3H, s), 3.81, 3.84 (1H, d, J = 12 Hz), 3.91, 3.94 (1H, d, J = 12 Hz), 5.50 (1H, broad s); mp 115–117 °C (dec).

(*S*)- and (*R*)-2-*N*-(*tert*-Butoxycarbonyl)amino-2-methyl-3-hydroxypropanoic Acid Methyl Ester (2). To a solution of (*S*)- or (*R*)-**1** (500 mg, 2.28 mmol) in 16 mL of benzene and 4.6 mL of methanol was added (trimethylsilyl)diazomethane (339 mg, 2.96 mmol, 1.48 mL of 2 M solution in hexane) in 2 mL of benzene dropwise. After the mixture was stirred for 30 min at room temperature, the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (20% ethyl acetate in hexane) to give a clear oil. (*S*)-2-*N*-(*tert*-butoxycarbonyl)amino-2-methyl-3-hydroxypropanoic acid methyl ester ((*S*)-**2**): 489 mg, (92%); ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.47 (3H, s), 3.78 (3H, s), 3.73–3.80 (1H, m), 3.97–4.01 (1H, m), 5.29 (1H, broad s). Anal. ($C_{10}H_{19}NO_5$) C, H, N. (*R*)-2-*N*-(*tert*-butoxycarbonyl)amino-2-methyl-3-hydroxypropanoic acid methyl ester ((*R*)-**2**): 413 mg (78%); ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s), 1.47 (3H, s), 3.79 (3H, s), 3.74–3.80 (1H, m), 3.97–4.01(1H, m), 5.28 (1H, broad s). Anal. Calcd for $C_{10}H_{19}NO_5$: C, 51.49; H, 8.21; N, 6.00. Found: C, 51.01; H, 8.26; N, 5.86.

(*S*)- and (*R*)-2-*N*-(*tert*-Butoxycarbonyl)amino-2-formylpropanoic Acid Methyl Ester (3). To a solution of oxalyl chloride (73 mg, 0.57 mmol, 0.27 mL of 2 M solution in dichloromethane) in 1 mL of dichloromethane cooled to −50 to −60 °C was added dimethyl sulfoxide (89 mg, 1.14 mmol) in 0.2 mL of dichloromethane. The mixture was stirred for 2 min followed by the addition of (*S*)- or (*R*)-**2** (122 mg, 0.52 mmol) in 0.4 mL of dichloromethane. The mixture was stirred at −50 to −60 °C for an additional 15 min, and then 0.36 mL of triethylamine (263 mg, 2.6 mmol) was added. Stirring was continued for another 5 min, and the mixture was warmed to room temperature. Water (2 mL) was added, and the two phases were separated. The aqueous layer was extracted with dichloromethane (3 × 4 mL). The organic layers were combined, washed with saturated sodium chloride solution (1 × 4 mL), and dried with anhydrous magnesium sulfate. The crude compound was subjected to chromatographic purification (20% ethyl acetate in hexane) to afford the product as a colorless oil. (*S*)-2-*N*-(*tert*-butoxycarbonyl)amino-2-formylpropanoic acid methyl ester ((*S*)-**3**): 95.6 mg (80%); ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 1.64 (3H, s), 3.81 (3H, s), 5.64 (1H, broad s), 9.57 (1H, s). Anal. ($C_{10}H_{17}NO_5$) C, H, N. (*R*)-2-*N*-(*tert*-butoxycarbonyl)amino-2-formylpropanoic acid methyl ester ((*R*)-**3**): 42.8 mg (36%); ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 1.64 (3H, s), 3.81 (3H, s), 5.65 (1H, broad s), 9.57 (1H, s). Anal. ($C_{10}H_{17}NO_5$) C, H, N.

(*S*)- and (*R*)-2-*N*-(*tert*-Butoxycarbonyl)amino-2-methyl-4-iodo-3-(*E*)-butenoic Acid Methyl Ester (4). Chromium(II) chloride (262 mg, 2.13 mmol) was suspended in 3 mL of tetrahydrofuran at room temperature under argon. A solution of (*S*)- or (*R*)-**3** (82 mg, 0.36 mmol) and iodoform (280 mg, 0.71 mmol) in 2 mL of tetrahydrofuran was added. The mixture was stirred at room temperature overnight. Water (10 mL) was added, and the aqueous layer was extracted with ether (3 × 10 mL). The combined organic layers were dried over magnesium sulfate, concentrated, and purified by silica gel chromatography (20% ethyl acetate in hexane) to provide the product as an oil. (*S*)-2-*N*-(*tert*-butoxycarbonyl)amino-2-methyl-4-iodo-3-(*E*)-butenoic acid methyl ester ((*S*)-**4**): 90.5 mg (72%); ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 1.57 (3H, s), 3.77 (3H, s), 5.24 (1H, broad s), 6.409, 6.446 (1H, d, J = 14.8 Hz), 6.793, 6.830 (1H, d, J = 14.8 Hz). HRMS, *m*/*z*, calcd for $C_{11}H_{18}INO_4Na$ $[M + Na]^+$, 378.017 28; found, 378.018 27. (*R*)-2-*N*-(*tert*-butoxycarbonyl)amino-2-methyl-4-iodo-3-(*E*)-butenoic acid methyl ester ((*R*)-**4**): 54.2 mg (43%); ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s), 1.58 (3H, s), 3.77 (3H, s), 5.24 (1H, broad s), 6.410, 6.446 (1H, d, J = 14.4 Hz), 6.795, 6.831 (1H, d, J = 14.4 Hz). HRMS, *m*/*z*, calcd for $C_{11}H_{19}INO_4$ $[M + H]^+$, 356.035 34; found, 356.036 30.

(*S*)- and (*R*)-2-Amino-2-methyl-4-iodo-3-(*E*)-butenoic acid (5). The *N*-Boc amino acid methyl ester (*S*)- or (*R*)-**4** (20 mg, 0.056 mmol) was suspended in 0.3 mL of 4 N HCl and heated to 120 °C for 20 min. The resulting homogeneous solution was then loaded on an ion-retardation resin (AG 11A8 50–100 mesh, BioRad) column in series with an alumina N Sep-Pak (Waters) and a C18 Sep-Pak (Waters) and eluted with water to provide the amino acid. (*S*)-2-Amino-2-methyl-4-iodo-3-(*E*)-butenoic acid ((*S*)-**5**): 11.6 mg, (75%); ^{1}H NMR (D_2O) δ 1.48 (3H, s), 6.39 (1H, d, J = 14 Hz), 6.74 (1H, d, J = 14 Hz). Anal. ($C_5H_8INO_2$) C, H, N. (*R*)-2-Amino-2-methyl-4-iodo-3-(*E*)-butenoic acid ((*R*)-**5**): 10.7 mg, (69%); ^{1}H

NMR (D_2O) δ 1.47 (3H, s), 6.39 (1H, d, $J = 14$ Hz), 6.75 (1H, d, $J = 14$ Hz). Anal. ($C_5H_8INO_2$) C, H, N.

(*S*)- and (*R*)-2-[*N*-(*tert*-Butoxycarbonyl)amino]-2-methyl-4-trimethylstannyl-3-(*E*)-butenoic Acid Methyl Ester (6). The protected amino acid (*S*)- or (*R*)-**4** (40 mg, 0.113 mmol) was dissolved in 2 mL of tetrahydrofuran and degassed with argon for 15 min. Hexamethylditin (74 mg, 0.225 mmol) and tetrakis(triphenylphosphine)palladium(0) (13 mg, 0.011 mmol) were added, and the solution was degassed for another 15 min. The mixture was heated to 50–60 °C for 2 h and cooled to room temperature. The solvent was removed under reduced pressure, and the crude product was purified by silica gel chromatography (ethyl acetate/hexane/triethylamine = 1:7:0.1%) to yield **6** as oil. (*S*)-2-[*N*-(*tert*-butoxycarbonyl)amino]-2-methyl-4-trimethylstannyl-3-(*E*)-butenoic acid methyl ester ((*S*)-**6**): 30.9 mg (70%); ^{1}H NMR ($CDCl_3$) δ 0.151 (9H, s), 1.44 (9H, s), 1.58 (3H, s), 3.75 (3H, s), 5.18 (1H, broad s), 6.05, 6.10 (1H, d, $J = 18.8$ Hz), 6.28, 6.33 (1H, d, $J = 18.8$ Hz). HRMS, *m*/*z*, calcd for $C_{14}H_{28}O_4N^{120}Sn$ $[M + H]^+$, 394.103 48; found, 394.103 71. (*R*)-2-[*N*-(*tert*-butoxycarbonyl)amino]-2-methyl-4-trimethylstannyl-3-(*E*)-butenoic acid methyl ester ((*R*)-**6**): 22.8 mg (52%); ^{1}H NMR ($CDCl_3$) δ 0.149 (9H, s), 1.43 (9H, s), 1.60 (3H, s), 3.75 (3H, s), 5.19 (1H, broad s), 6.05, 6.10 (1H, d, $J = 18.8$ Hz), 6.28, 6.33 (1H, d, $J = 18.8$ Hz). HRMS, *m*/*z*, calcd for $C_{14}H_{28}O_4N^{120}Sn$ $[M + H]^+$, 394.103 48; found, 394.104 72.

Radiosynthesis. The preparation of radioiodinated (*S*)-**5** and (*R*)-**5** was carried out with no-carrier-added [^{123}I]sodium iodide or [^{131}I]sodium iodide and the appropriate stannyl precursor according to the method of Van Dort.[17] Typically, 80 μg (204 nmol) of (*S*)-**6** or (*R*)-**6** in 200 μL of ethanol was added to [^{123}I]NaI (in 20–50 μL of 0.1 N NaOH, 1–3 mCi) or [^{131}I]NaI (in 2–3 μL of 0.1 N NaOH, 1–3 mCi) in a 1.5 mL Wheaton V-vial. Then 50 μL of 0.4 N HCl (aq) and 50 μL of 3% H_2O_2 (aq, w/v) were added to the V-vial. The vial was shaken occasionally at room temperature for 20 min. The reaction was quenched by the addition of aqueous sodium metabisulfite (50 μL, 250 mg/mL) followed by the addition of saturated aqueous sodium bicarbonate (500 μL). After extraction with dichloromethane (3 × 0.5 mL), the combined organic layers were dried by blowing argon through at 65 °C. The residue was cooled to room temperature, 400 μL of 4 N HCl was added, and the mixture was heated at 110 °C for 20 min. The product (*S*)- or (*R*)-[$^{123/131}$I]**5** was obtained by purification through a 7 mm × 120 mm column of ion-retardation resin (AG11A8 50–100 mesh) in series with an Alumina N SepPak and a C-18 SepPak eluted with saline (0.9% sodium chloride in water). The eluting fractions containing radioactivity were collected and used in cell and rodent studies. The identity of the radiolabeled product was confirmed by comparing the R_f of the radioactive product visualized with radiometric TLC with the R_f of the authentic nonradioactive form of the compound visualized with ninhydrin stain ($R_f = 0.6$, 4:1:1 CH_3CN/H_2O/MeOH). In all radiosyntheses, the only peak present on radiometric TLC analysis corresponded to (*S*)-**5** or (*R*)-**5**, and the radiochemical purity of the product exceeded 99%. The isolated radiochemical yields were determined using a dose calibrator.

Amino Acid Uptake and Inhibition Assays. The amino acid uptake assays were performed with cultured rat 9L gliosarcoma cells as described previously.[12,13] The data from these studies were normalized as percent uptake relative to standard per 1×10^6 cells. The cell uptake data were analyzed with one- and two-way ANOVAs using GraphPad Prism software (San Diego, CA).

Rodent Biodistribution in Tumor Bearing Rats. Cultured 9L cells were implanted into the brains of male Fischer 344 rats (125–175 g) for biodistribution studies as described previously.[12,13,18] A total of 15 animals were used in the (*S*)-[^{123}I]**5** study (5 at each time point), and 13 animals were used in the (*R*)-[^{131}I]**5** study (5 at 30 min, 4 at both 60 and 120 min) in separate biodistribution studies. Intravenous doses of 15–30 μCi (*S*)-[^{123}I]**5** or (*R*)-[^{131}I]**5** were administered in 0.1–0.3 mL of sterile saline. The biodistribution studies were performed at 10–12 days after implantation, and the data were analyzed with two-way ANOVAs using GraphPad Prism software.

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