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Synthesis and evaluation of [^{123}I] labeled iodovinyl amino acids *syn*-, *anti*-1-amino-3-[2-iodoethenyl]-cyclobutane-1-carboxylic acid, and 1-amino-3-iodomethylene-cyclobutane-1-carboxylic acid as potential SPECT brain tumor imaging agents

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

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

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Abstract—*syn*- and *anti*-1-amino-3-[2-iodoethenyl]-cyclobutane-1-carboxylic acid (*syn*-, *anti*-IVACBC **16**, **17**) and their analogue 1-amino-3-iodomethylene-cyclobutane-1-carboxylic acid (*gem*-IVACBC **18**) were synthesized and radioiodoinated with [^{123}I] in 34–43% delay-corrected yield. All these amino acids entered 9L gliosarcoma cells primarily via L-type transport in vitro with high uptake of 8–10% ID/1 × 10^6 cells. Biodistribution studies of [^{123}I]**16**, **17** and **18** in rats with 9L gliosarcoma brain tumors demonstrated high tumor to brain ratios (4.7–7.3:1 at 60 min post-injection). In this model, *syn*-, *anti*-, and *gem*-[^{123}I]IVACBC are promising radiotracers for SPECT brain tumor imaging.

The development of radiotracers for detecting cancer in patients is a major focus of radiopharmaceutical research. A number of classes of compounds that accumulate preferentially in neoplastic tissues have been investigated for this purpose, including metabolically based radiotracers such as amino acids. Amino acids are important biological substrates in virtually all biological processes and are required nutrients for cell growth. 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). The amino acids developed for oncologic imaging can be divided into two major categories, naturally occurring amino acids and their structurally similar analogues, and non-natural amino acids. One potential advantage of non-natural amino acids over naturally occurring amino acids is their increased metabolic stability. Additionally, non-natural amino acids may not participate effectively in protein synthesis. These properties can potentially simplify the analysis of tracer kinetics with non-natural amino acids.

Non-natural amino acid PET tracers *anti*-1-amino-3-[^{18}F]fluorocyclobutane-1-carboxylic acid (*anti*-[^{18}F]FACBC)[1,2] and *syn*- and *anti*-1-amino-3-[^{18}F]fluoromethyl-cyclobutane-1-carboxylic acid (*syn*- and *anti*-[^{18}F]FMACBC)[3] have been prepared in this lab and have shown great potential in imaging brain and prostate cancer.[4–6] The radionuclides used for PET and SPECT have complementary properties. While PET has higher temporal and spatial resolution, SPECT radionuclides possess certain advantages. Most commonly used SPECT radionuclides, including iodine-123 and technecium-99*m*, are available without an onsite cyclotron. SPECT radionuclides have longer half-lives (e.g., 13.2 h for iodine-123 and 6 h for technecium-99*m*) than most widely used PET radionuclides (e.g., 110 min for fluorine-18 and 20 min for carbon-11). The longer half-lives of most SPECT radionuclides facilitate chemical synthesis, allow for the study of processes that occur over longer time courses, and are suitable for remote distribution of the radiolabeled product to sites that do not have radiosynthetic capabilities. There are

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* Corresponding author. Tel.: +1 404 727 9366; fax: +1 404 727 3488; e-mail: mgoodma@emory.edu

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currently fewer radiolabeled amino acids suitable for SPECT than for PET, with [^{123}I]IMT[7] representing the most widely used radiolabeled amino acid for SPECT.

In an effort to develop the radiotracers for SPECT tumor imaging, we report here the syntheses of *syn*-, *anti*-1-amino-3-[2-iodoethenyl]-cyclobutane-1-carboxylic acid (*syn*-, *anti*-IVACBC **16**, **17**), 1-amino-3-iodomethylene-cyclobutane-1-carboxylic acid (*gem*-IVACBC **18**), which are analogues of FACBC and FMACBC, their [^{123}I] radiolabeling and biological evaluation with a rat 9L gliosarcoma cell line and in Fischer rats with 9L tumors implanted intracranially (Fig. 1).

The syntheses of *syn*- and *anti*-IVACBC stannylated precursors for radiolabeling were prepared in a series of synthetic steps starting from allylbenzylether (**1**), which is shown in Scheme 1. The intermediates, the *syn*- and *anti*-1-[*N*-(*tert*-butoxycarbonyl)amino]-3-hydroxymethyl-cyclobutane-1-carboxylic acid *tert*-butyl esters (**2**,**3**), were prepared in 7 steps according to Martarello's method.[3] The alcohol isomers were transformed to the corresponding aldehydes (**4**,**5**) by Swern oxidation.[8] In the key synthetic step, reaction of **4** or **5** with iodoform in tetrahydrofuran using chromium(II) chloride as a catalyst gave exclusively *trans* (*E*) isomers of **6** and **7**[9–12] as determined by ^{1}H NMR. The stannyl labeling precursors *syn*- and *anti*-1-[*N*-(*tert*-butoxycarbonyl)amino]-3-[2-trimethylstannylethenyl]-cyclobutane-1-carboxylic acid *tert*-butyl esters **8** and **9** were prepared from vinyl iodides **6** and **7**, respectively, using hexamethylditin and catalytic tetrakis(triphenylphosphine) palladium(0).[12,13]

The protected *gem*-IVACBC stannylated precursor for radiolabeling was prepared starting from epibromohydrin (**10**). The key intermediate, a 5:1 mixture of *syn*/*anti*-5-(3-benzyloxycyclobutane)hydantoins (**11**), was prepared in 4 steps as described previously,[1] and hydrolyzed at 120 °C with 3 N NaOH to the *syn*/*anti*-1-amino-3-benzyloxycyclobutane-1-carboxylic acids. Subsequent *N*-protection with di-*tert*-butyl dicarbonate and *tert*-butyl esterification with *tert*-butyl 2,2,2-trichloroacetimidate followed by Pd^0 catalyzed debenzylation[3] gave the *syn*/*anti*-1-[*N*-(*tert*-butoxycarbonyl)amino]-3-hydroxy-cyclobutane-1-carboxylic acid *tert*-butyl esters (**12**). The alcohols were transformed to the corresponding ketone (**13**) by Swern oxidation followed by reaction with $CHI_3/CrCl_2$ to form protected *gem*-IVACBC (**14**) which was treated with $(SnMe_3)_2/Pd^0(PPh_3)_4$ to afford 1-[*N*-(*tert*-butoxycarbonyl)amino]-3-trimethylstannylmethylene-cyclobutane-1-carboxylic acid *tert*-butyl ester (**15**) as the radiolabeling precursor[12] (Scheme 2).

The ^{127}I references of *syn*-, *anti*-, and *gem*-IVACBC **16**, **17**, and **18** were prepared from the *N*-Boc amino acid

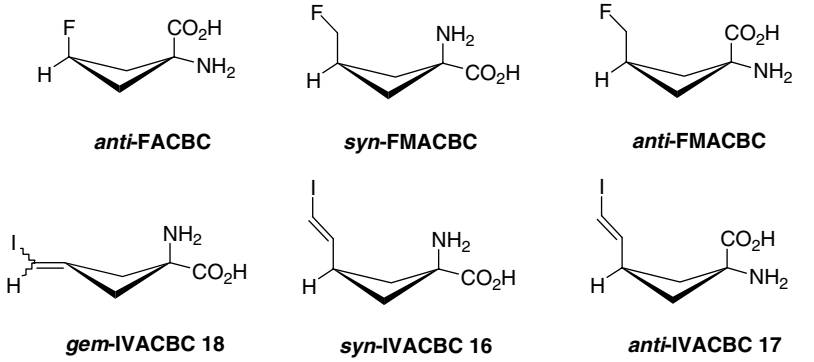
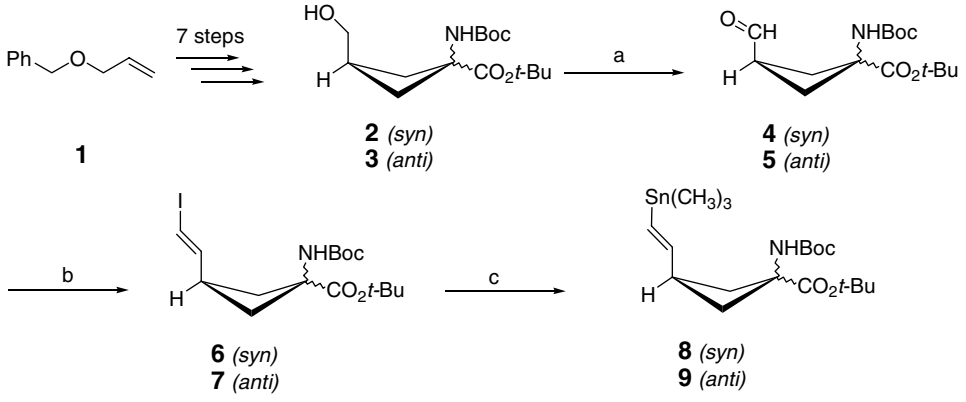
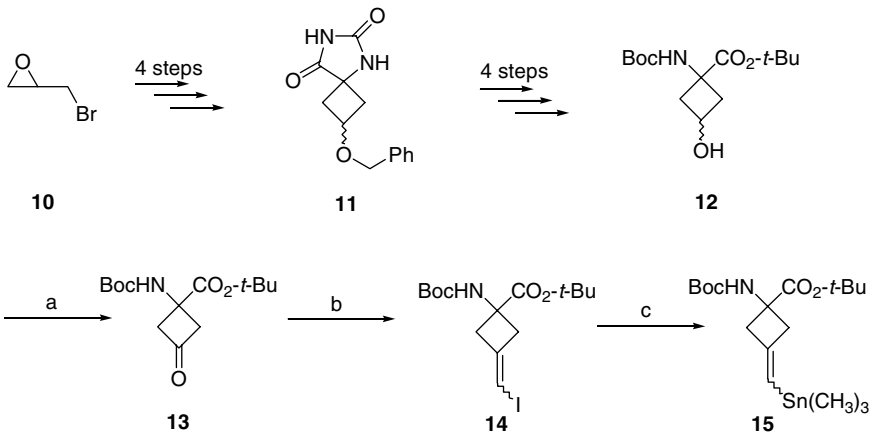


Figure 1. Structures of FACBC and its fluoro and iodovinyl analogues.



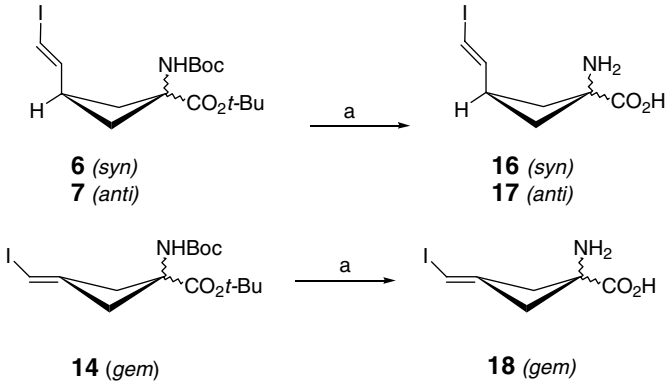
Scheme 1. Syntheses of *syn*- and *anti*-IVACBC stannylated precursors **8** and **9**. Reagents and conditions: (a) $(COCl)_2$, DMSO, DCM, −50 °C then Et_3N, 79% (**4**); 64% (**5**); (b) CHI_3, $CrCl_2$, THF, 0 °C—rt, 59% (**6**); 60% (**7**); (c) $(CH_3)_3Sn–Sn(CH_3)_3$, $Pd^0(PPh_3)_4$, THF, 50–60 °C, 26% (**8**); 18% (**9**).



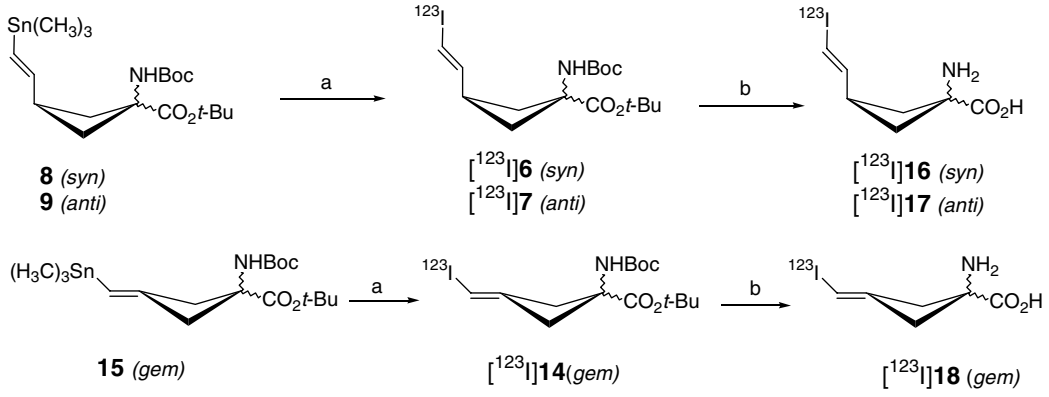
Scheme 2. Synthesis of *gem*-IVACBC stannylated precursor **15**. Reagents and conditions: (a) $(COCl)_2$, DMSO, DCM, −50 °C then Et_3N, 92% (**13**); (b) CHI_3, $CrCl_2$, THF, 0 °C—rt, 27% (**14**); (c) $(CH_3)_3Sn–Sn(CH_3)_3$, $Pd^0(PPh_3)_4$, THF, 50–60 °C, 76% (**15**).

tert-butyl esters **6**, **7** and **14**, respectively, by treatment of trifluoroacetic acid (TFA) and purified by ion-retardation resin chromatography[12] (Scheme 3).

Radioiodinated *syn*-, *anti*-[^{123}I]IVACBC **16**, **17**, and *gem*-[^{123}I]IVACBC **18** were prepared using no-carrier-added (NCA) sodium[^{123}I]iodide under oxidizing conditions followed by deprotection with TFA (Scheme 4). The radioiodinated products were purified by ion-retardation resin chromatography[12]. The procedure required approximately 100 min with decay-corrected yields (d.c.y.) of 41.9 ± 18.3% (n = 17, *syn*-[^{123}I]IVACBC **16**), 43.4 ± 23.0% (n = 12, *anti*-[^{123}I]IVACBC **17**) and 33.7 ± 18.2% (n = 7, *gem*-[^{123}I]IVACBC **18**) in over 99% radiochemical purity as measured by radiometric TLC. Based on 200 nmol of tin precursor utilized for labeling, and approximate yields of 1 mCi of product, the minimum specific activities for *syn*-, *anti*-, and



Scheme 3. Syntheses of *syn*-, *anti*-, and *gem*-IVACBC **16**, **17**, and **18**. Reagents and conditions: (a) TFA, DCM, rt, 78% (**16**); 71% (**17**); 68% (**18**).



Scheme 4. Radiosyntheses of *syn*-, *anti*-, and *gem*-IVACBC **16**, **17**, and **18**. Reagents and conditions: (a) [^{123}I]NaI, H_2O_2, H^+, rt; (b) TFA, DCM, 85 °C, 42% (**16**); 43% (**17**); 34% (**18**).

gem-[^{123}I] **16**, **17**, and **18** are estimated at 5 mCi/μmol. The final product was not assayed for the presence of tin.

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 and transport mechanism. 2-Amino-bicyclo[2.2.1]-heptane-2-carboxylic acid (BCH) (10 mM) and *N*-methyl-α-aminoisobutyric acid (MeAIB) (10 mM) were used as L- and A-type amino acid transport inhibitors, respectively. The results of these amino acid uptake assays are depicted in Figure 2. In the absence of inhibitors, [^{123}I]IVACBC **16**, **17**, and **18** showed high levels of intracellular accumulation, 8.4–9.9% of the initial dose per million cells (% ID/1 × 10^6 cells) in 9L gliosarcoma cells. In the presence of BCH, 92.3–97.5% of inhibition was observed in all these compounds compared to controls ($p < 0.001$ in all cases, one-way ANOVA). In contrast, no significant uptake inhibition occurred with MeAIB for **16** and **18** and 14.3% inhibition for **17** relative to controls. These results demonstrate that compounds **16**, **17**, and **18** are selective substrates for L-type amino acid transport in 9L gliosarcoma cells in vitro.

The in vivo biodistribution studies were performed in Fischer rats with 9L tumors implanted intracranially. The radioactivity in tumors and in normal tissues of tumor-bearing rats ($n = 5$ each time point) was calculated at 30, 60, 120 min post-injection (p.i.) and normalized as percent injected dose per gram tissue (% ID/g). The uptake of radioactivity of [^{123}I]IVACBC **16**, **17**, and **18** in tumor and brain is presented in Figure 3. The experiments showed that these amino acids had rapid and prolonged accumulation in tumors, ranged from 0.83 to 1.85% ID/g and was significantly higher than in normal brain tissue ($p < 0.001$ at all time points, one-way ANOVA). The uptake in normal brain tissue was less than 0.4% ID/g at all time points for all these compounds thus the tumor to normal brain uptake ratios were 7.3:1, 6.5:1, and 4.7:1 at 60 min p.i. for [^{123}I]IVACBC **16**, **17**, and **18**, respectively. Low uptake was found in heart, liver, lung, bone, and thyroid. The low thyroid uptake with all radiotracers indicates that free iodide was not generated during the time course of the study.

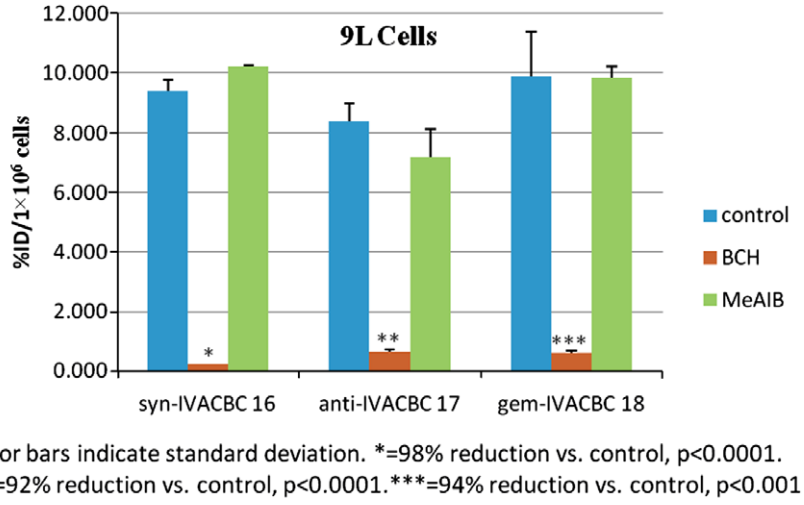


Figure 2. 9L cell uptake and inhibition assays with [^{123}I]**16**, [^{123}I]**17**, and [^{123}I]**18**.

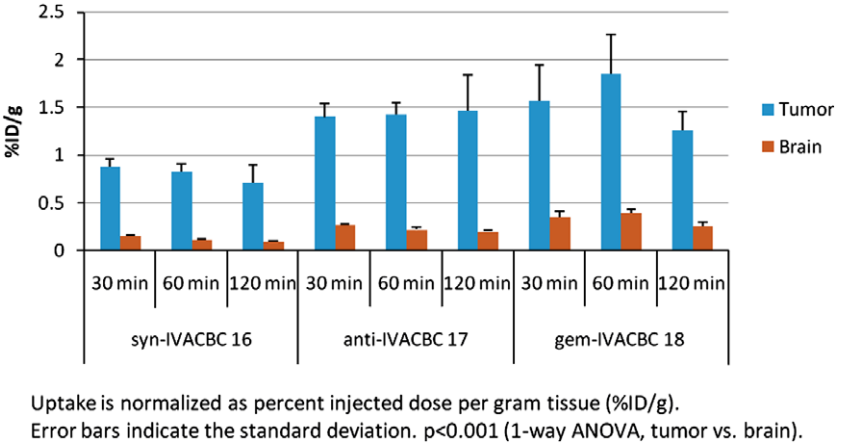


Figure 3. Radioactive uptake in tumor and brain of 9L tumor-bearing Fisher rats with [^{123}I]IVACBC **16**, **17**, and **18**.

The uptake of radioactivity of [^{18}F] labeled *anti*-FACBC,[2] *syn*-FMACBC[3], and *anti*-FMACBC[3] in tumors at 60 min p.i. were 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.[2,3] Thus, the characteristics of candidate compounds **16**, **17**, and **18** are comparable with *anti*-FACBC, *syn*-FMACBC, and *anti*-FMACBC, by entering 9L cells via L-type transport system in vitro and showing high levels of uptake in 9L brain tumors in vivo in comparison to normal brain.

In summary, three new SPECT tumor imaging agents, *syn*-, *anti*-, and *gem*-IVACBC **16**, **17**, and **18** have been synthesized, [^{123}I] labeled and biologically evaluated. All these compounds demonstrated high levels of tumor uptake in vitro and in vivo in a 9L rat gliosarcoma brain tumor model and they are L-type amino acid transporter substrates. These results are comparable with those of *anti*-FACBC, *syn*-FMACBC, and *anti*-FMACBC in the same animal model, which support the candidacy of *syn*-, *anti*-, and *gem*-IVACBC **16**, **17**, and **18** as promising SPECT brain tumor imaging agents.

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