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Supporting Information

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Supporting Information

Imparting Multivalency to a Bifunctional Chelator: A Scaffold Design for Targeted PET Imaging Probes

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I. General Experimental Details

General Methods and Materials

All reactions were carried out under an argon atmosphere in degassed dried solvents. Commercially available starting compounds were purchased from Sigma-Aldrich (St. Louis, MO) and used directly without further purification unless otherwise noted. Silica gel 60 (70-230 mesh, Merck) was used for column chromatography. Analytical thin-layer chromatography (TLC) was performed using Merck 60 F254 silica gel (precoated sheets, 0.2 mm thick) (Lawrence, KS). The spectra of ^{1}H NMR, ^{13}C NMR were recorded on a Varian 400 or 500 MHz spectrometer; chemical shifts are expressed in ppm relative to TMS (0 ppm), or chloroform (7.26 ppm). (MALDI) mass spectra were acquired on an Applied Biosystems Voyager-6115 mass spectrometer. Bulk solvent removal was done by rotary evaporation under reduced pressure, and trace solvent was removed by vacuum pump. Cross-bridge cyclam and α-bromoglutaric acid-1-tertbutylester-4-benzyl ester **3** (scheme 1) were synthesized according to published procedures [1] [2]. 1,4,8,11-tetraazacyclotetradecane was purchase from Macrocylic Inc. (Dallas, TX).

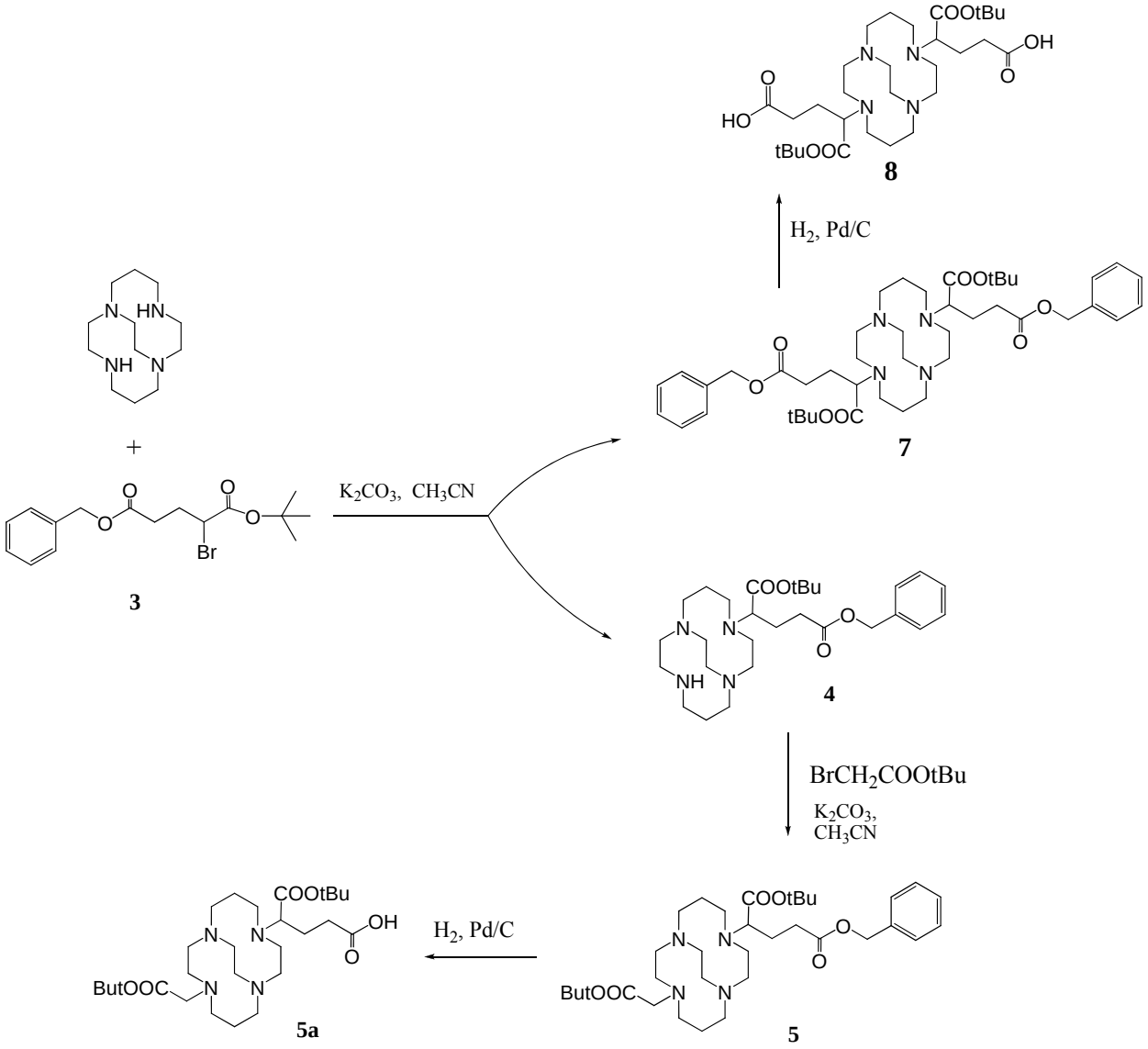
HPLC condition

High performance liquid chromatography (HPLC) was performed on a Waters 600 Multisolvent Delivery System equipped with a Waters 2996 Photodiode Array a detector. The mobile phase was H_2O with 0.1% TFA (solvent A) and acetonitrile with 0.1% TFA (solvent B). The analytical analysis was performed on an XTerra RP18 Column with a gradient of 0% B to 100 % B in 50

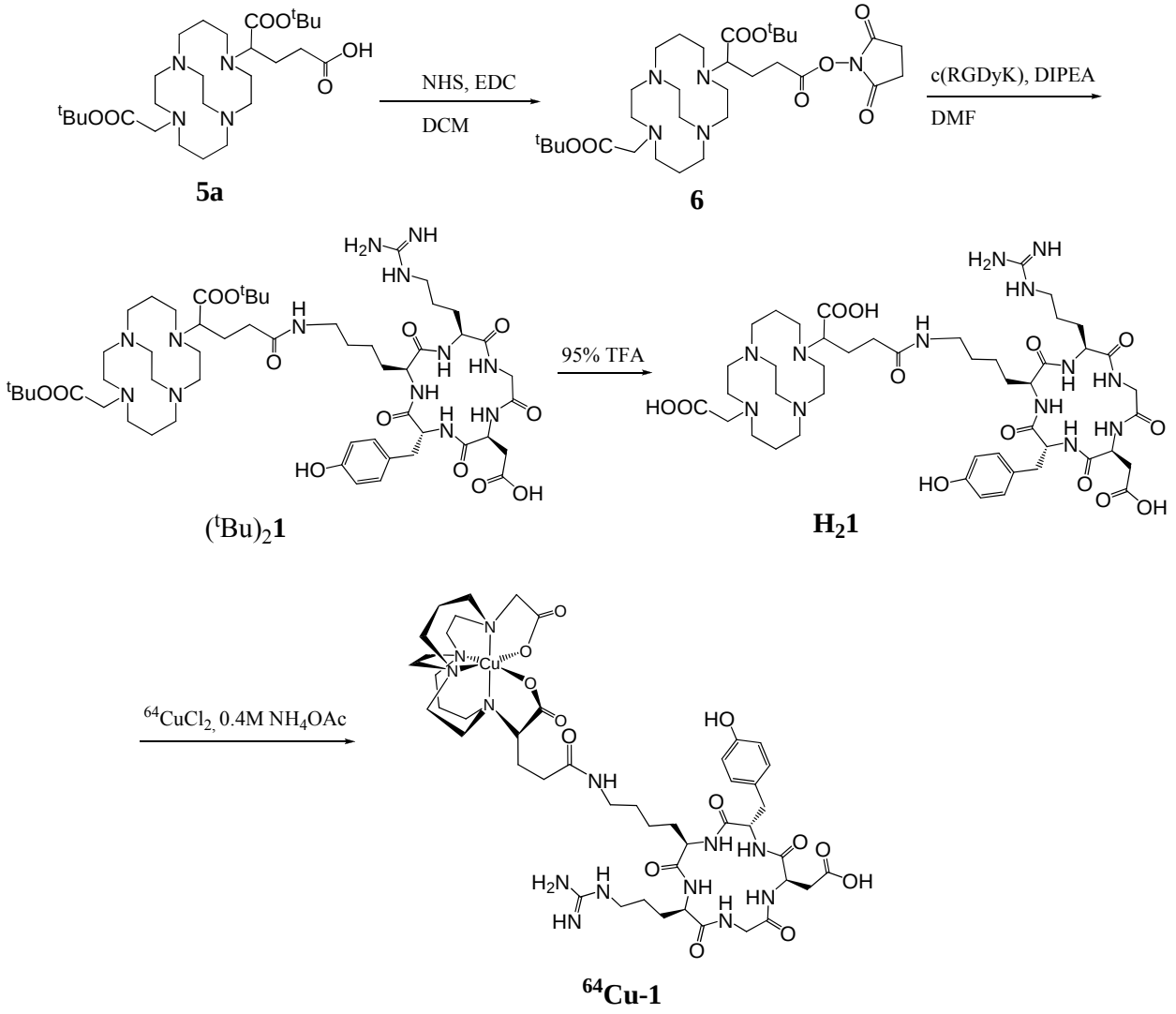
min at a flow rate of 1.0 mL/min. The HPLC separation was performed on a semi-preparative XTerra RP18 Column with a gradient of 0% B to 100 % B in 50 min at a flow rate of 4.0 mL/min.

II. Compound Synthesis and Characterization

Scheme S1.

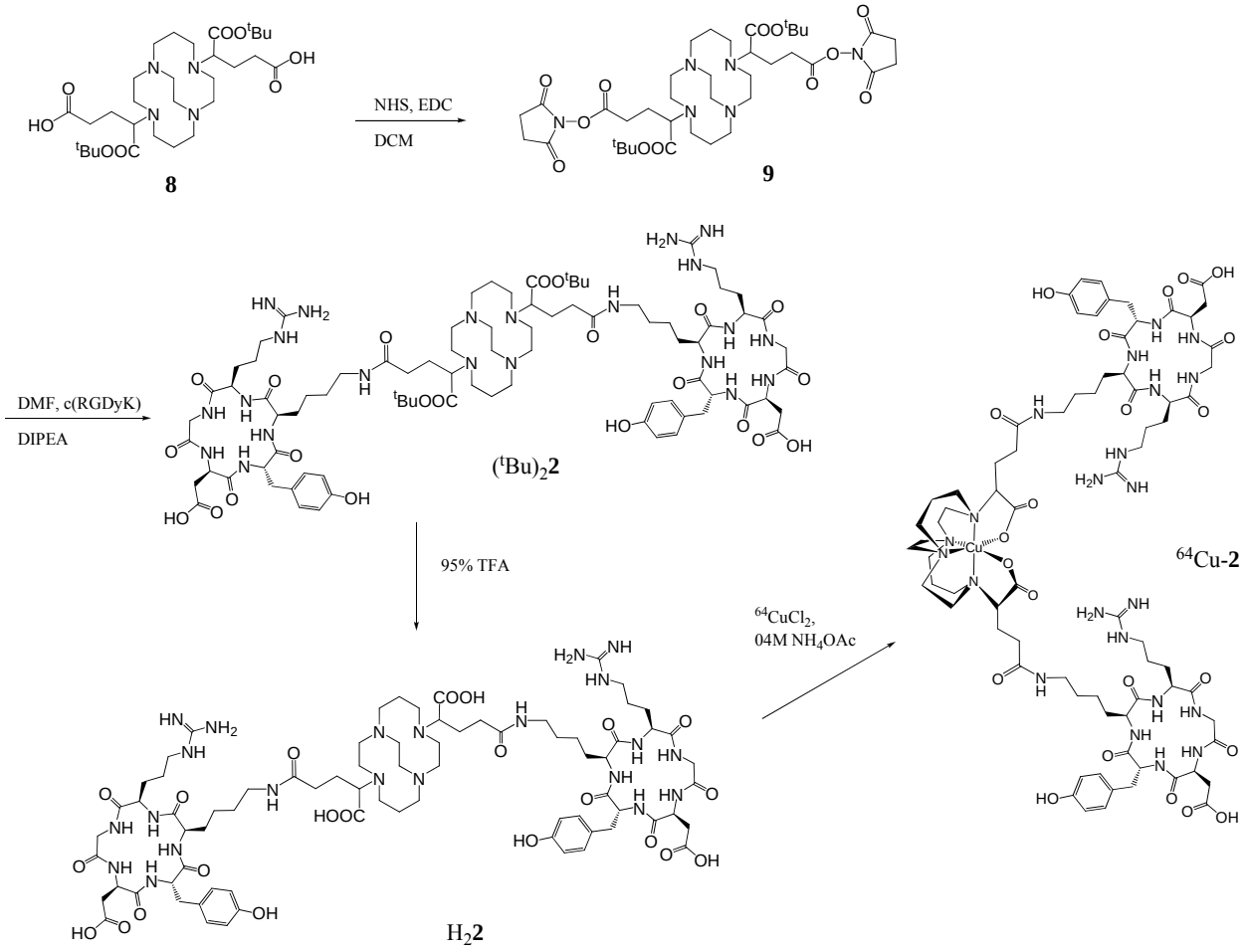


Scheme S2.



5a **6** $(^{t}Bu)_2$**1** $\mathbf{H_2}$**1** ^{64}**Cu-1**

Scheme S3.



5-benzyl 1-tert-butyl 2,2'-(1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-diyl)dipentane-dioate (4; Scheme S1). To a suspension of cross-bridge cyclam (230 mg, 1.03mmol) and K_2CO_3 (0.57 g, 4.1 mmol) in 5 mL of anhydrous acetonitrile at 0°C, compound **3** (384 mg, 1.08 mmol) in 2 mL of anhydrous MeCN was added dropwise. After the addition, the reaction was maintained at 0°C for 5 h, and then allowed to proceed at room temperature (r. t.) for 3 days. The solids were removed by filtration and washed with chloroform (2 × 20 mL). The combined filtrate was concentrated under vacuum and purified by column chromatography (silica gel, 60-230 mesh) using 10:1 $CHCl_3$/MeOH to 9:1 EtOAc/isopropyl amine (R_f = 0.43) for elution. Compound **4** was obtained as a sticky oil (286 mg; Yield: 55%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.42 (s, 9H, *t*-butyl), 1.78-1.95 (m, 1H), 1.98-2.13 (m, 1H), 2.14-2.21 (m, 1H), 2.34-3.25 (m,

26H), 5.09 (s, 2H, CH_2-Ph), 7.32 (s, 5H, Ph); ^{13}C NMR ($CDCl_3$,100 MHz) δ 25.43, 26.17, 27.48, 28.56, 31.73, 48.92, 49.16, 49.38, 52.04, 53.94, 54.11, 57.90, 59.15, 60.40, 62.04, 66.50, 81.24, 128.45, 128.78, 136.16, 172.59, 173.27; MALDI-TOF/MS: 503.71 [M + H^+].

5-benzyl 1-tert-butyl 2-(11-(2-tert-butoxy-2-oxoethyl)-1,4,8,11-tetraazabicyclo[6.6.2]hexadecan-4-yl)pentanedioate (5; Scheme S1). To 2 mL of **4** (100 mg, 0.20 mmol) in acetonitrile, K_2CO_3 (120mg, 0.87 mmol) was added followed by methyl chloroacetate (39 mg, 0.20 mmol) in 2 mL of acetonitrile. The resulting mixture was stirred at r. t. for 2 days. After removal of the solids by filtration and evaporation of the solvent, the residue was redissolved in chloroform and washed thoroughly with water. The organic layer was dried over sodium sulfate. Removal of the solvent under vacuum afforded the crude product as a brown oil, which was purified by column chromatography (silica gel, 60-230 mesh) using 10:1 $CHCl_3$/MeOH to 9:1 EtOAc/isopropyl amine (R_f = 0.60) for elution. Compound **5** was obtained as a pale yellow sticky oil (56 mg; Yield: 92%): ^1H NMR ($CDCl_3$, 500 MHz) δ 1.46 (s, 18H, *t*-butyl), 1.73-3.20 (m, 29H), 3.58 (m, 1H), 3.90 (m, 1H), 5.12 (s, 2H, CH_2-Ph), 7.36 (s, 5H, Ph); MALDI-TOF/MS: 617.12 [M + H^+].

Compound (^{t}Bu)$_2$1 (Scheme S2). To a solution of **5** (100 mg, 0.16 mmol) in 5 mL of mixture solvent THF/H_2O (1:1) was added portion wise 10 mg of 10% Pd/C. The suspension was shaken in a hydrogenator (Parr, Moline, Illionis) at r. t. for 12 h under an H_2 atmosphere (60 psi). After removal of the solids, evaporation of solvent and lyophilize to remove the remaining water, compound **5a** was obtained as a white foam in nearly quantitative yield. Then a mixture of compound **5a** (100mg, 0.19 mmol), *N*-hydroxysuccinimide (43mg, 0.38 mmol) and EDC·HCl (73 mg, 0.38 μmol) in 1 mL of dry acetonitrile was stirred under N_2 for 24 hours. After removal of the solvent under reduced pressure, the residue was dissolved in chloroform (1 mL) and was washed 3 times (3 × 2 mL) by water promptly. Upon the removal of chloroform under vacuum, the solution was lyophilized to afford a pale yellow solid **6**. Compound **6** was used directly for the next reaction without further purification. A cyclic RGD peptide [c(RGDyK)] (10 mg, 16 μmol) was then mixed with **6** (12 mg, 19 μmol) in 500 μL of anhydrous DMF, to which 30 μL of N, N-diisopropylethylamine (DIPEA) was added (*ca.* 10 equivalent to RGD). The mixture was stirred at room temperature for two days. After removal of the solvent, the crude product was purified by semi-preparative reverse-phase HPLC. The collected fractions from multiple

runs were pooled and lyophilized to afford (^{t}Bu)$_2$**1** as a white foam (5.0 mg; Yield: 29%). MALDI-TOF/MS: 1127.88 [M+H$^+$].

Compound H$_2$1 (Scheme S2). Compound (^{t}Bu)$_2$**1** (5 mg, 4.4 μmol) was dissolved in 95% of TFA and stirred at room temperature for 12 h. After evaporation of the solvent, the residue was purified by semi-preparative reverse-phase HPLC. The collected fractions were from multiple runs were pooled and lyophilized to afford a white solid at quantitative yield. MALDI-TOF/MS: 1015.03 [M+H$^+$].

5-Benzyl 1-tert-butyl 2-(1,4,8,11-tetraazabicyclo[6.6.2]hexadecan-4-yl)pentanedioate (7; Scheme S1). To a suspension of cross-bridge cyclam (180 mg, 0.80 mmol) and K_2CO_3 (1.2 g, 8.68 mmol) in 5 mL of anhydrous of acetonitrile was added compound **3** (630 mg, 1.76 mmol) in 2 mL of anhydrous acetonitrile was added. The reaction was stirred at r. t. for 24 h and then the temperature was elevated to and kept at 50 °C for 24 h. The solids were removed by filtration and washed with chloroform (2 × 20 mL). The combined filtrate was concentrated under vacuum and purified by column chromatography (silica gel, .60-230 mesh) using 10:1 $CHCl_3$/MeOH to 9:1 EtOAc/isopropyl amine (R_f = 0.84) for elution. Compound **7** was obtained as a sticky oil (390.6 mg; Yield: 45%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.44 (s, 18H, *t*-butyl), 1.79-1.90 (m, 2H), 1.92-2.80 (m, 2H), 2.23-3.20 (m, 28H), 3.59-3.68 (m, 1H), 3.72-3.85 (m, 1H), 5.11 (s, 4H, CH_2-Ph), 7.34 (s, 10H, Ph); ^{13}C NMR ($CDCl_3$,100 MHz) δ 25.29, 28.38, 30.91, 50.32 (br), 51.67 (br), 53.34 (br), 55.89 (br), 62.43, 66.75, 82.69, 128.49, 128.63, 128.85, 135.99, 171.22, 172.65; MALDI-TOF/MS: 779.60 [M+H$^+$].

4,4'-(1,4,8,11-tetraazabicyclo[6.6.2]hexadecane-4,11-diyl)bis(5-tert-butoxy-5-oxopentanoic acid) (8; Scheme S1). To a solution of **7** (100 mg, 0.13 mmol) in 5 mL of THF/H_2O (2:1) was added portionwise 10 mg of 10% Pd/C. The suspension was shaken at r. t. for 16 h under a hydrogen atmosphere (60 psi) in a hydrogenator. After removal of the solids, evaporation of the solvents under vacuum afforded compound **8** as a white solid in quantitative yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.40 (s, 18H, *t*-butyl), 1.56-2.00 (m, 8H), 2.34-2.33 (m, 4H), 3.30-2.73 (m, 22H) ; ^{13}C NMR ($CDCl_3$, 100 MHz) δ 24.58, 27.36, 27.65, 31.95, 50.27 (br), 51.52 (br), 54.21(br), 62.62, 83.94, 173.72, 177.99; MALDI-TOF/MS: 599.60 [M+H$^+$].

Compound (^{t}Bu)$_2$2 (Scheme S3). A mixture of compound **8** (10.0 mg, 16.7 μmol), *N*-hydroxysuccinimide (7.6 mg , 66.8 μmol) and EDC·HCl (12.8 mg, 66.8 μmol) in 500 μL of dry acetonitrile was stirred under N_2 for 24 h. After removal of the solvent under reduced pressure, the residue was redissolved in chloroform (1 mL) and then washed 3 times with water (3 × 2 mL). After evaporation of chloroform, the residue was dried by a lyophilizer to yield a pale yellow solid. The NHS-activated ester (compound **9**; Scheme S3) was used directly for the RGD conjugation without further purification. Cyclic RGD peptide [c(RGDyK)] (10 mg, 16 μmol) was mixed with **9** (2.4 mg, 4 μmol) in 200 μL of anhydrous DMF, to which 30 μL of N, N-diisopropylethylamine (DIPEA) was added (*ca.* 10 equivalent to RGD). The mixture was stirred at r. t. for 24 h under a nitrogen atmosphere. After evaporation of the solvent under vacuum, the crude product was purified by semi-preparative reverse-phase HPLC. The collected fractions of multiple runs were pooled and lyophilized to afford 2.2 mg of (^{t}Bu)$_2$**2** as a white powder (30%). MALDI-TOF/MS: 1800.12 [M+H$^+$].

Compound $H_2$2 (Scheme S3). Compound (^{t}Bu)$_2$**2** (2 mg, 1.1 μmol) was dissolved in 95% of TFA and stirred at r. t. for 12 h. After evaporation of the solvent, the residue was purified by semi-preparative reverse-phase HPLC. The collected fractions of multiple runs were pooled and lyophilized to afford a white solid (H_2**2**) in quantitative yield. MALDI-TOF/MS: 1688.73 [M+H$^+$].

III. Radiochemical Procedures

Radiolabeling of $H_2$1 or $H_2$2 with ^{64}Cu: To a 1.5 mL vial containing 5 μg of H_2**1** or H_2**2** in 200 μL of 0.4 M NH_4OAc (pH = 6.5) solution, 2 – 3 mCi of $^{64}CuCl_2$ in 0.1 M HCl was added. The reaction mixture was shaken and incubated at 75ºC for 0.5 h. Then, 5 μL of 5 mM diethylenetriaminepentaacetic acid (DTPA) was added to the reaction mixture, which was allowed to incubate for another 5 min (DTPA was used to remove non-specifically bound or free ^{64}Cu from the ^{64}Cu-labeled **1** or **2**). The purification of ^{64}Cu-**1** or ^{64}Cu-**2** was carried out by passing the mixture through a Sep-Pak C-18 plus cartridge. After thorough rinsing the cartridge two times with water, the ^{64}Cu-labeled product was eluted out by pure ethanol. The product was

firstly analyzed by radio-TLC and then by radio-HPLC to determine the radiochemical purity of the product.

Serum Stability: An aliquot (*ca.* 40 μCi) of ^{64}Cu-**1** or ^{64}Cu-**2** was added into each of 12 vials containing 100 μL of rat serum. The vials were incubated at 37 ºC in a water bath. At each time point (1, 2, 4 and 24 h), 420 μL of ethanol was added to three of the vials to precipitate the serum proteins. After high speed (14,000 rpm) centrifugation for 5 min, the supernatant was removed and then the pellet was resuspended with 240 μL of 80% ethanol. The suspension was centrifuged again and the supernatant was collected. The supernatants were pooled and then analyzed by radio-HPLC. The dislodged ^{64}Cu from ^{64}Cu-**1** or ^{64}Cu-**2** if any is assumed to be associated with the serum proteins.

Cell Integrin Receptor-Binding Assay: The $\alpha_\upsilon\beta_3$ integrin-binding affinities of ^{64}Cu-**1** and ^{64}Cu-**2**. were determined by a competitive cell-binding assay using ^{125}I-echistatin (PerkinElmer) as the $\alpha_\upsilon\beta_3$-specific radioligand. The experiments were performed on U87MG human glioblastoma cells by a previously reported method [3, 4]. Briefly, U87MG cells were grown in Dulbecco's modified Eagle medium (DMEM, low glucose) supplemented with 10% (v/v) fetal bovine serum (FBS) at 37ºC with 5% CO_2. Suspended U87MG cells in binding buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 0.1% bovine serum albumin) were seeded on multi-screen DV plates (Millipore) with 2×10^5 cells per well and then incubated with ^{125}I-echistatin (10,000 cpm/well) in the presence of increasing concentrations (0 – 5,000 nM) of c(RGDyK) peptide conjugates for 2 h. The final volume in each well was maintained at 200 μL. At the end of incubation, the unbound ^{125}I-echistatin was removed by filtration and then three times of rinsing with cold binding buffer. The filters were collected and the radioactivity was measured using a γ-counter. The best-fit IC_{50} values (inhibitory concentration where 50% of the ^{125}I-echistatin bound on U87MG cells are displaced) of c(RGDyK), H_2**1**, and H_2**2** were calculated by fitting the data with nonlinear regression using GraphPad Prism (GraphPad Software, Inc.). Experiments were duplicated with quintuplicate samples. The results are shown in Figure S1.

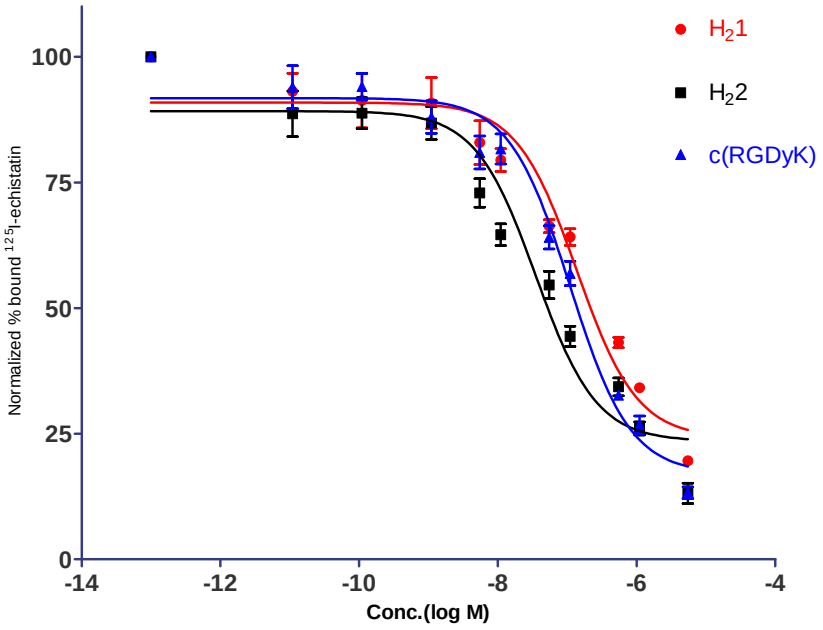


Figure S1. The $\alpha_v\beta_3$ binding affinities of H_2**1** and H_2**2** measured by a competitive cell-binding assay using U87MG cells where ^{125}I-echistation was employed as $\alpha_v\beta_3$-specific radioligand. The IC_{50} values of c(RGDyK), H_2**1,** and H_2**2**, were determined to be 110, 139 and 35 nM, respectively (n = 5).

IV. In vivo PET-CT Imaging and Biodistribution

Tissue Culture and Animal Model: All animal studies were performed in compliance with guidelines set by the UT Southwestern Institutional Animal Care and Use Committee. The PC-3 cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA). PC-3 cells were cultured in T-media (Invitrogen, Carlsbad, CA) at 37 °C in an atmosphere of 5% CO_2 and were passaged at 75 % confluence in P150 plates. T-media was supplemented with 5% Fetal Bovine Serum (FBS) and 1 × Penicillin/Streptomycin. PC-3 cells were harvested from monolayer using PBS and trypsin/EDTA, and suspended in T-media with 5% FBS. The cell suspension was then mixed 1:1 with Matrigel™ and injected subcutaneously (2.5×10^6 cells per injection, injection volume 100 μL) into both front flanks of SCID mice. After injection, animals

were monitored three times a week by general observations. The tumor was noticed to grow in the first week and allowed to grow three weeks to reach a palpable size for microPET-CT imaging and post-PET biodistribution studies. Tumor volume (mm^3) was calculated using the ellipsoid formula ($\pi/6 \times$ length $\times$ width $\times$ depth).

MicroPET-CT Imaging: Small animal PET-CT imaging studies were performed on a Siemens Inveon PET-CT Multimodality System (Siemens Medical Solutions Inc., Knoxville, TN, USA). When the prostate cancer xenografts reached the similar size of approximate 100 mm^3, the tumor-bearing mice were randomized into four groups (n = 3) for the in vivo evaluation of ^{64}Cu-**1,** ^{64}Cu-**2**, ^{64}Cu-**1** with co-injection of c(RGDyK), and ^{64}Cu-**2** with co-injection of c(RGDyK). The dose of c(RGDyK) used for blocking was 10 mg/kg. The injected dose was kept at 100 μCi of ^{64}Cu-activity in 100 μL PBS; and the injected molar amounts of ^{64}Cu-**1** and ^{64}Cu-**2** were maintained at the same level by decay correction.

Ten minutes prior to imaging, the animal was anesthetized using 3% Isofluorane at room temperature until stable vitals were established. Once the animal was sedated, the animal was placed onto the imaging bed under 2% Isofluorane anesthesia for the duration of the imaging. The microCT imaging was acquired at 80 kV and 500 μA with a focal spot of 58 μm. The total rotation of the gantry was 360^o with 360 rotation steps obtained at an exposure time of approximately 235 ms/frame. The images were attained using a CCD readout of 4096 × 3098 with a bin factor of 4 and an average frame of 1. Under low magnification the effective pixel size was 103.03 μm. Total microCT scan time was approximately 6 minutes. CT images were reconstructed with a down sample factor of 2 using Cobra Reconstruction Software. The PET imaging was acquired directly following the acquisition of CT data. The PET tracers were injected intravenously via the tail vein. Static PET scans were performed at 1 h, 4 h, and 24 h post injection (p.i.) for 15 min. PET images were reconstructed using Fourier Rebinning and Ordered Subsets Expectation Maximization 3D (OSEM3D) algorithm. Reconstructed CT and PET images were fused and analyzed using the Siemens Inveon Research Workplace (IRW) software. For quantification, regions of interest were placed in the areas expressing the highest radiotracer activity as determined by visual inspection. The tissues examined include the left and right tumors, the heart, liver, lung, kidney, and muscle. The resulting quantitative data was expressed as percent injected dose per gram of tissue (%ID/g).

Post-PET Biodistribution: Immediately after the 24 h imaging, the mice were sacrificed and organs of interest were removed, weighed, and counted by a γ-counter. Standards were prepared and counted along with the samples to calculate the percent injected dose per gram (%ID/g) and percent injected dose per organ (%ID/organ).

Table S1. Uptake of Cu-**1** in major organs and tumor determined by PET quantification. Data are presented as %ID/g ± s.d. (n = 3)

Cu-**1**	1 h	1 h (blockade)	4 h	4 h (blockade)	24 h	24 h (blockade)
Lung	0.44 ± 0.04	0.20 ± 0.08	0.17 ± 0.04	0.07 ± 0.03	0.17 ± 0.04	0.02 ± 0.00
Liver	1.37 ± 0.12	0.52 ± 0.17	1.17 ± 0.21	0.46 ± 0.19	0.50 ± 0.05	0.20 ± 0.00
Kidney	2.33 ± 0.15	1.77 ± 0.57	1.80 ± 0.00	1.09 ± 0.27	0.96 ± 0.14	0.47 ± 0.13
Spleen	Not Visible	Not Visible	Not Visible	Not Visible	Not Visible	Not Visible
Heart	0.48 ± 0.05	0.21 ± 0.03	0.12 ± 0.01	0.03 ± 0.00	0.15 ± 0.01	0.03 ± 0.01
Stomach	0.79 ± 0.24	0.25 ± 0.01	0.42 ± 0.08	0.11 ± 0.01	0.31 ± 0.12	0.07 ± 0.00
Muscle	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00	0.01 ± 0.02	0.00 ± 0.00	0.00 ± 0.00
Tumor (left)	1.93 ± 0.15	0.29 ± 0.02	1.87 ± 0.35	0.18 ± 0.07	1.17 ± 0.21	0.12 ± 0.02
Tumor (right)	1.97 ± 0.06	0.33 ± 0.08	1.83 ± 0.21	0.18 ± 0.06	1.03 ± 0.06	0.12 ± 0.01

Table S2. Uptake of Cu-**2** in major organs and tumor determined by PET quantification. Data are presented as %ID/g ± s.d. (n = 3)

Cu-**2**	1 h	1 h (blockade)	4 h	4 h (blockade)	24 h	24 h (blockade)
Lung	0.67 ± 0.01	0.39 ± 0.03	0.48 ± 0.06	0.23 ± 0.01	0.33 ± 0.04	0.14 ± 0.05
Liver	3.10 ± 0.17	1.50 ± 0.14	2.65 ± 0.07	1.45 ± 0.21	2.40 ± 0.10	1.20 ± 0.28
Kidney	4.13 ± 0.29	3.10 ± 0.28	3.10 ± 0.00	1.80 ± 0.28	2.33 ± 0.21	1.40 ± 0.14
Spleen	2.20 ± 0.43	Not Visible	2.2 ± 0.10	Not Visible	2.6 ± 0.10	Not Visible
Heart	0.66 ± 0.02	0.39 ± 0.03	0.44 ± 0.08	0.23 ± 0.01	0.24 ± 0.03	0.12 ± 0.04
Stomach	1.50 ± 0.44	0.57 ± 0.05	1.20 ± 0.14	0.58 ± 0.01	1.20 ± 0.10	0.37 ± 0.05
Muscle	0.47 ± 0.19	0.10 ± 0.11	0.17 ± 0.01	0.03 ± 0.03	0.12 ± 0.07	0.01 ± 0.01
Tumor (left)	2.90 ± 0.35	0.70 ± 0.05	2.35 ± 0.35	0.44 ± 0.02	1.70 ± 0.20	0.31 ± 0.14
Tumor (right)	2.93 ± 0.21	0.72 ± 0.04	2.45 ± 0.07	0.46 ± 0.06	1.73 ± 0.21	0.29 ± 0.13

Table S3. Uptake of Cu-**1** and Cu-**2** in major organs and tumor determined by post-PET biodistribution. Data are presented as %ID/g ± s.d. (n = 3).

	Cu-**1**		Cu-**2**	
	24 h	24 h (blockade)	24 h	24 h (blockade)
Lung	0.29 ± 0.04	0.08 ± 0.01	1.07 ± 0.07	0.62 ± 0.06
Liver	0.31 ± 0.06	0.16 ± 0.02	2.40 ± 0.06	1.70 ± 0.07
Kidney	1.12 ± 0.19	0.65 ± 0.18	2.73 ± 0.03	1.62 ± 0.20
Spleen	0.91 ± 0.15	0.27 ± 0.06	3.20 ± 0.12	0.98 ± 0.08
Heart	0.12 ± 0.02	0.03 ± 0.01	0.49 ± 0.05	0.29 ± 0.01
Stomach	0.50 ± 0.08	0.05 ± 0.01	1.21 ± 0.16	0.34 ± 0.01
Small intestine	0.38 ± 0.04	0.06 ± 0.01	1.39 ± 0.16	0.43 ± 0.06
Large intestine	0.53 ± 0.27	0.07 ± 0.00	1.17 ± 0.37	0.43 ± 0.09
Bone	0.08 ± 0.06	0.05 ± 0.04	0.28 ± 0.03	0.10 ± 0.02
Brain	0.06 ± 0.08	0.00 ± 0.00	0.05 ± 0.00	0.03 ± 0.00
Tumor (left)	1.51 ± 0.09	0.18 ± 0.01	1.72 ± 0.11	0.51 ± 0.05
Tumor (right)	1.26 ± 0.14	0.19 ± 0.03	1.87 ± 0.11	0.47 ± 0.02

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