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Synthesis of Tc-99m labeled glucosamino-Asp-cyclic(Arg-Gly-Asp-D-Phe-Lys) as a potential angiogenesis imaging agent

Byung Chul Lee,[a] Hyun Ju Sung,[a] Ji Sun Kim,[a] Kyung-Ho Jung,[b] Yearn Seong Choe,[b] Kyung-Han Lee[b] and Dae Yoon Chi[a,*]

[a]*Department of Chemistry, Inha University, 253 Yonghyundong, Namgu, Inchon 402-751, Republic of Korea*
[b]*Department of Nuclear Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-dong Kangnam-ku, Seoul 135-710, Republic of Korea*

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Abstract—Angiogenesis imaging agents for single photon emission computed tomography (SPECT) play a role in diagnosing tumor-induced angiogenesis as well as tumor metastasis. We synthesized and evaluated radiolabeled RGD glycopeptides by incorporation of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$. ^{99m}Tc labeled glucosamino-D-c(RGDfK) ([^{99m}Tc]**2**) was prepared in 90–93% radiochemical yields (decay corrected). In vitro cell binding assays demonstrated selective binding [^{99m}Tc]**2** to human umbilical vein endothelial (HUVE) cells, with inhibition of binding to 37.3% of control levels by 10 μM of cold authentic compounds. In addition, [^{99m}Tc]**2** was shown to have high binding affinity to purified $\alpha_v\beta_3$ integrin (IC_{50} = 1.5 nM). These results suggest that these radiolabeled RGD glycopeptides may have value for non-invasive assessment of angiogenesis.

1. Introduction

Integrins are a superfamily of heterodimeric transmembrane glycoproteins that consist of 19 α-subunits and 8 β-subunits known to date. These receptors play a major role in cellular adhesion, migration, and signal transduction.[1–3] Among them, $\alpha_v\beta_3$ integrin is involved in cell–cell and cell–matrix interaction, and plays a particularly important role in tumor metastasis and angiogenesis.[4,5] In addition to being implicated in cancer progression, $\alpha_v\beta_3$ integrin expression is also involved in improvement and healing of ischemic lesions.[6,7] Blocking of $\alpha_v\beta_3$ integrins leads to apoptosis of endothelial cells and inhibition of tumor blood vessel formation.[8] The binding region in the $\alpha_v\beta_3$ integrin contains the tripeptide amino acid sequence: arginine (R), glycine (G), and aspartic acid (D) which is commonly called RGD.[9–14] As such, most angiogenesis imaging agents that have been developed contain a cyclic form of this RGD sequence (cRGD) as shown in Figure 1. These include cRGD glycopeptides such as cRGD[^{125}I]yV, cRGD[^{125}I]yK(SAA), and [^{18}F]galacto-cRGDfK, as $\alpha_v\beta_3$ integrin antagonists labeled with iodine-125 or fluorine-18.[15–18] More recently, an RGD radiotracer composed of 1,4,7,10-tetraazadodecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (DOTA) or hydrazinonicotinamide (HYNIC) conjugate chelated with ^{111}In, ^{90}Y, or ^{99m}Tc has been introduced.[19,20]

The design of new radiolabeled derivatives containing a RGD moiety should include the following criteria: low hepatic uptake, high selectivity toward $\alpha_v\beta_3$ integrin, and preparation by simple radiolabeling method. With these criteria, we synthesized new radiolabeled RGD analog. To increase hepatic clearance, a glucosamino group was inserted in the target molecule, which could be used to improve the pharmacokinetics.[16–18,21,22] Linkage of the prosthetic group and a glucosamino group via an Asp residue to Lys of the RGD moiety should have little influence on the selectivity of RGD binding to $\alpha_v\beta_3$ integrin. Radiolabeled cRGD derivatives were prepared using incorporation of tricarbonyl technetium-99m to NO_2 ligand attached to the amino terminal of an Asp residue.

Keywords: Angiogenesis; Radiolabeled RGD; Cyclic RGD; AlphV beta3 integrin; Technetium-99m.
* Corresponding author. Tel.: +82 32 860 7686; fax: +82 32 867 5604; e-mail: dychi@inha.ac.kr

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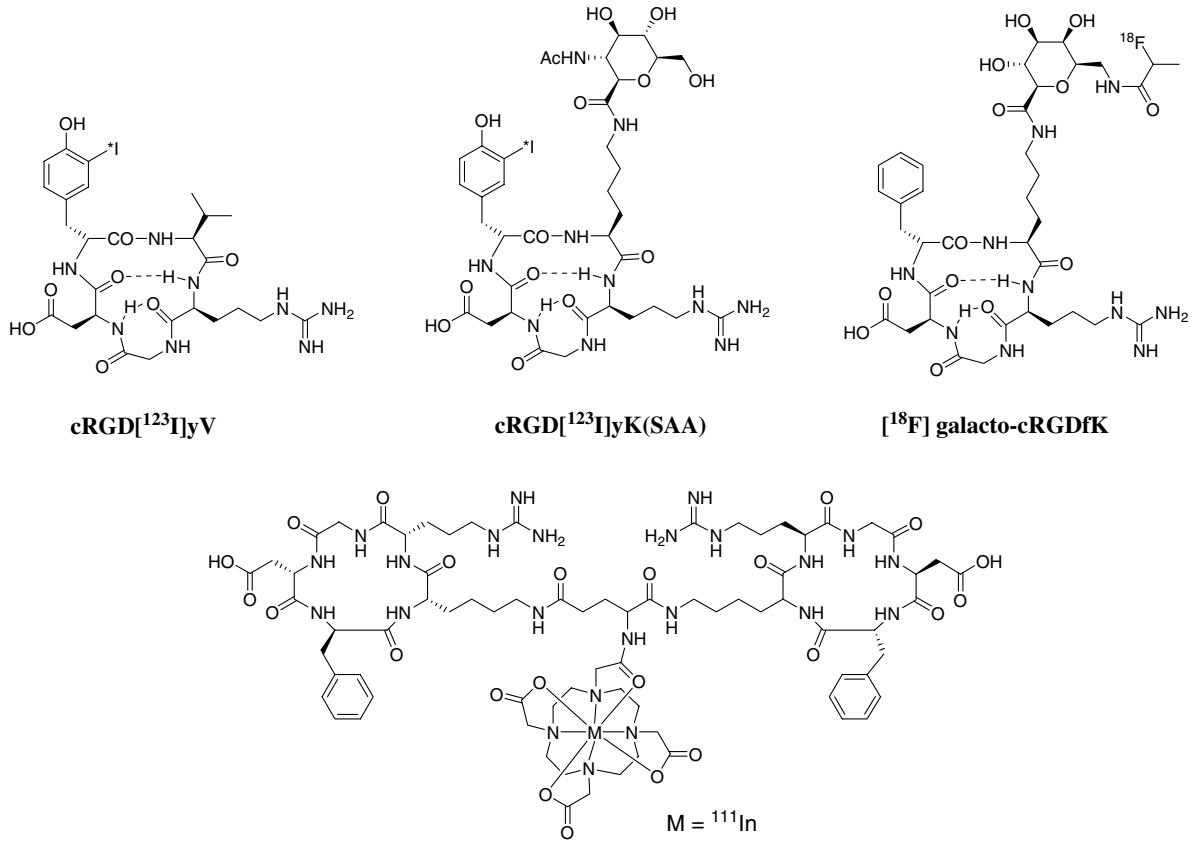


Figure 1. Radiolabeled cRGD derivatives.

Radiolabeled RGD analog was synthesized to allow labeling with technetium-99m as SPECT tracers. Single photon emitting tracers have advantages because of the wide availability of SPECT cameras. Moreover, technetium-99m is desirable due to its ideal physical properties (141 keV, $t_{1/2}$ = 6 h) and ready availability.[23,24]

Tricarbonyl technetium-99m is an attractive core for the introduction of ^{99m}Tc into biomolecules because of its high chemical stability and small size. $[^{99m}Tc(H_2O)_3(CO)_3]^+$ can be readily generated from $^{99m}TcO_4^-$ and CO gas in the presence of $NaBH_4$.[25–28] Therapeutic radionuclides such as the β-emitting rhenium-186 and rhenium-188 $[^{186/188}Re(H_2O)_3(CO)_3]^+$ could be applied by the coordination with the same class of ligands used in $[^{99m}Tc(H_2O)_3(CO)_3]^+$ reagent.[29]

In this work, we designed a technetium-99m labeled RGD glycopeptide ([^{99m}Tc]Tc labeled glucosamino-D-c(RGDfK), [^{99m}Tc]**2**) as a SPECT agent for angiogenesis imaging (Fig. 2).

2. Results and discussion

2.1. Chemical synthesis

To improve pharmacokinetics of the target compound, we introduced applicative *N*-D-glucosamine to the compound as a glucose moiety.[16,17] Treatment of *N*-acetyl-D-glucosamine with acetyl chloride in methanol, followed by direct benzylation at 3-OH, 4-OH, and 6-OH, resulted in formation of methylation compound **5** at 1-OH, which avoids the reduction to a pentose form

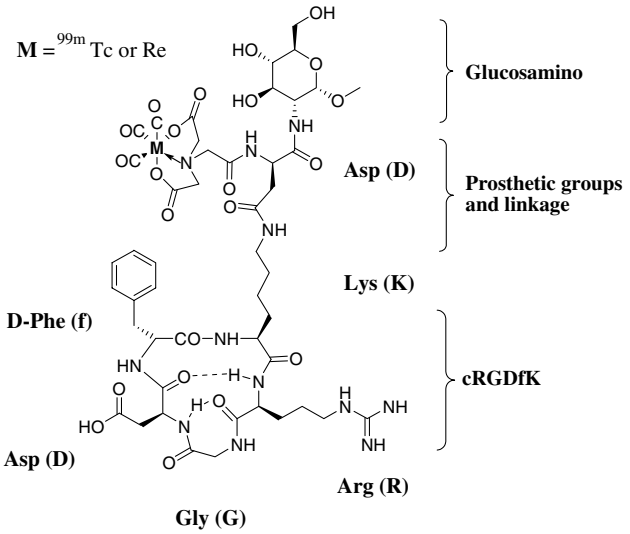


Figure 2. Structures of target compounds. Target compounds are divided into three parts: glucosamino moiety, prosthetic group with linkage, and cyclic RGD moiety.

under the condition of the following steps as shown in Scheme 1.

Mono acetyl protected amino group of compound **5** was further protected with *tert*-butyloxycarbonyl (Boc) group in dry THF in the presence of 4-dimethylaminopyridine (DMAP), so that *N*-acetyl group can be readily removed under mild condition. Free amine **7** could not be obtained by direct hydrolysis of *N*-acetamide **5**. This methodology has already been applied for the synthesis of amide-linked amino acid.[30–34] The N-deacetylation in NaOMe followed by acidic hydrolysis in trifluoroacetic acid (TFA) of **6** afforded the free amine **7** in 95% yield. The amide condensation between compound **7** and Fmoc-Asp(O^{t}Bu)-OH gave product **8** in 71% yield.

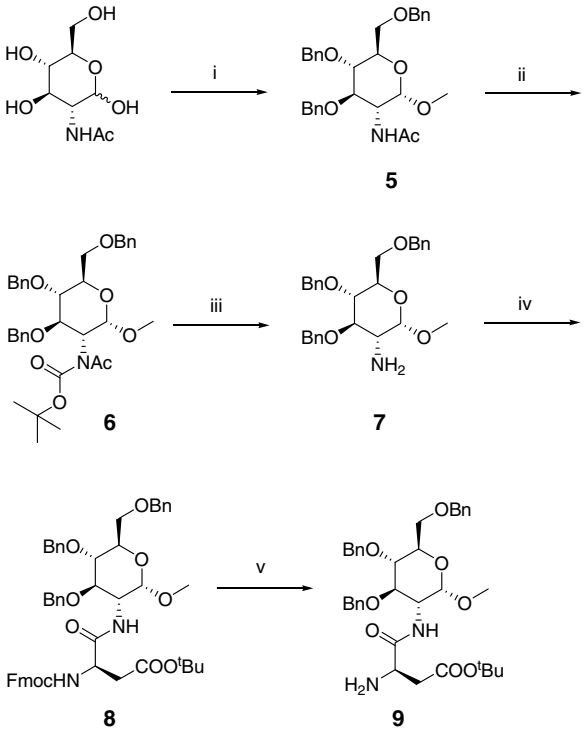
After removal of the 9-fluorenylmethoxycarbonyl (Fmoc) group, the amino group of compound **9** was linked with a technetium pocket, *N*-α-bis(benzyloxycarbonylmethyl)-glycine-OH (**4**) from glycine *tert*-butyl ester and benzyl bromoacetate, as shown in Scheme 2.

The small size of *N*-bis(hydroxycarbonylmethyl)-[^{99m}Tc]tricarbonyl led us to design and synthesize a technetium-99m labeled cRGD derivative as an $\alpha_v\beta_3$ integrin antagonist. *tert*-Butyl esters of compound **10** were subjected to acid hydrolysis to afford acid compound **11**. It has been shown that the addition of Lys residue to the RGD derivative has no influence on its affinity to the $\alpha_v\beta_3$ integrin.[15] The glucosamino aspartic derivative **11** was further linked with cyclic-R(Pbf)-G-D(O^{t}Bu)-f-K-NH_2 using an amide bond condensation as shown in Scheme 3. The protecting groups of compounds **12**—2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) and *tert*-butyl groups at the side chain of Arg and Asp, respectively—were removed by the treatment of a mixture of trifluoroacetic acid/$HSCH_2CH_2SH$/H_2O (95:2.5:2.5) to obtain the desired compounds **13**.

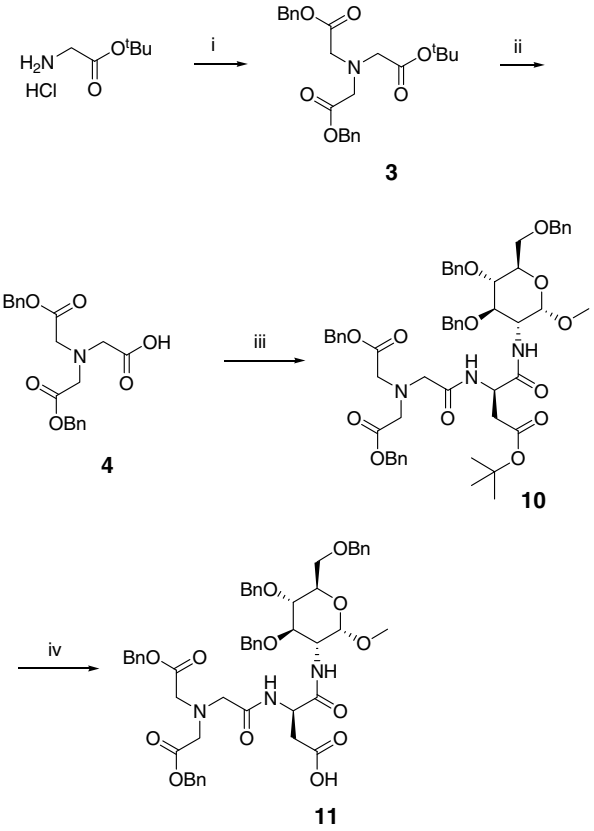
The precursor **14** was prepared from the compound **13** in the presence of 10% palladium on charcoal within a hydrogen atmosphere (Scheme 4). The debenzylation was confirmed by HPLC. After the mixture was filtered through a short plug filled with C-18 silica gel (3 cm), the solvent was evaporated in vacuo, the resulting compound **14** was purified by reverse phase HPLC.

Re coordination reaction with the precursor **14** was performed in a water/methanol mixture (1:1) at 65 °C using $(NEt_4)_2[ReCl_3(CO)_3]$.[27,35,36] The reaction was also monitored by HPLC until the compound **14** peak disappeared.

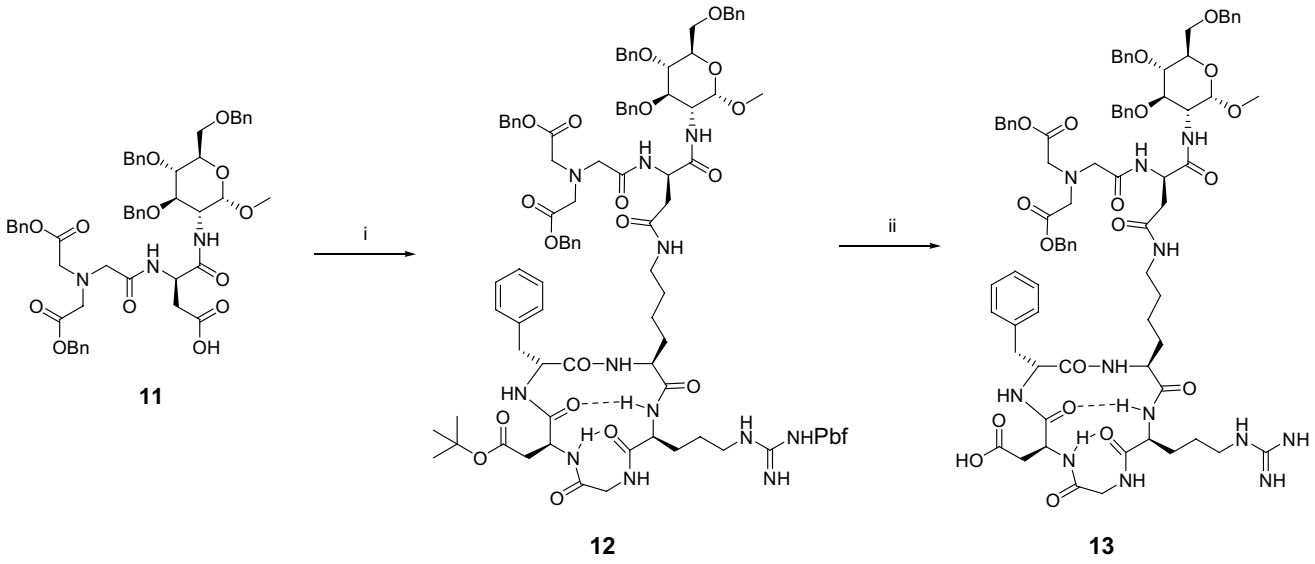
Incorporation of tricarbonyl technetium-99m into the compound **14** using $[^{99m}Tc(H_2O)_3(CO)_3]^+$ was carried out in aqueous media. The precursor *fac*-$[^{99m}Tc(H_2O)_3(CO)_3]^+$ was readily prepared using a known method.[25–27] The radiochemical yield of [^{99m}Tc]**2** was 90–92%.



Scheme 1. Reagents and conditions: (i) 1—MeOH, AcCl, 0–80 °C, 1 h, 2—BnBr, NaOH/DMF, rt, 12 h; (ii) Boc_2O, DMAP, THF, 80 °C, N_2, 18 h; (iii) 1—NaOMe/MeOH, rt, N_2, 2.5 h, 2—TFA/CH_2Cl_2, rt, 2 h; (iv) Fmoc-Asp(O^{t}Bu)-OH, HOBT, TBTU, DIEA, DMF, rt, N_2, 16 h; (v) 20% piperidine/DMF, rt, 20 min.



Scheme 2. Reagents and conditions: (i) benzyl bromoacetate, DIEA, CH_2Cl_2, 0 °C, 6 h; (ii) TFA/CH_2Cl_2, rt, 2 h; (iii) 9, HOBT, TBTU, DIEA, DMF, rt, N_2, 12 h; (iv) TFA/CH_2Cl_2, rt, 2 h.



Scheme 3. Reagents and conditions: (i) cyclic-Arg(Pbf)-Gly-Asp(O′Bu)-D-Phe-Lys-NH_2, HOBT, TBTU, DIEA, DMF, rt, N_2, 12 h; (ii) trifluoroacetic acid/$HSCH_2CH_2SH$/water (95:2.5:2.5), rt, 24 h.

2.2. In vitro endothelial cell binding assay of [^{99m}Tc]2, and cRGD[^{125}I]yV

Accumulation of radioactivity in human umbilical vein endothelial (HUVE) cells and ECV304 cells was evaluated in a paired format to compare [^{99m}Tc]**2** uptake levels with that of cRGD[^{125}I]yV. The binding characteristics of our radiotracers were therefore compared to that of cRGD[^{125}I]yV, a radiotracer known to exhibit high affinity and selective binding to $\alpha_v\beta_3$ integrin.[15] HUVE cells were incubated with [^{99m}Tc]**2**, or cRGD[^{125}I]yV for 60 min, then counted for bound radioactivity. Results were expressed as % cell bound fractions of added radioactivity calculated from standards.

As shown in Figure 3, [^{99m}Tc]**2** was shown to have significantly higher uptake levels at 60 min than that of cRGD[^{125}I]yV ([^{99m}Tc]**2**/cRGD[^{125}I]yV = 2.9%ID (2.3:0.8)). This tendency was also seen in HUVEC-derived, ECV304 cells ([^{99m}Tc]**2**/cRGD[^{125}I]yV = 3.3%ID (1.8:0.55)). Thus, endothelial binding of [^{99m}Tc]**2** was higher than cRGD[^{125}I]yV, despite a more complicated structure.

2.3. In vitro stability studies

Percentage of the remaining [^{99m}Tc]**2** was 98% after 240 min and over 95% even after 12 h when incubated in human serum at 37 °C and analyzed by radio-TLC, indicating a high in vitro stability of radiotracer as shown in Figure 5. This result means that the technetium core of complex as well as RGD fraction is very stable as similar result of technetium core itself which was previously reported.[37]

2.4. Binding competition of labeled RGD ([^{99m}Tc]2) with cRGDyV or authentic compound (1)

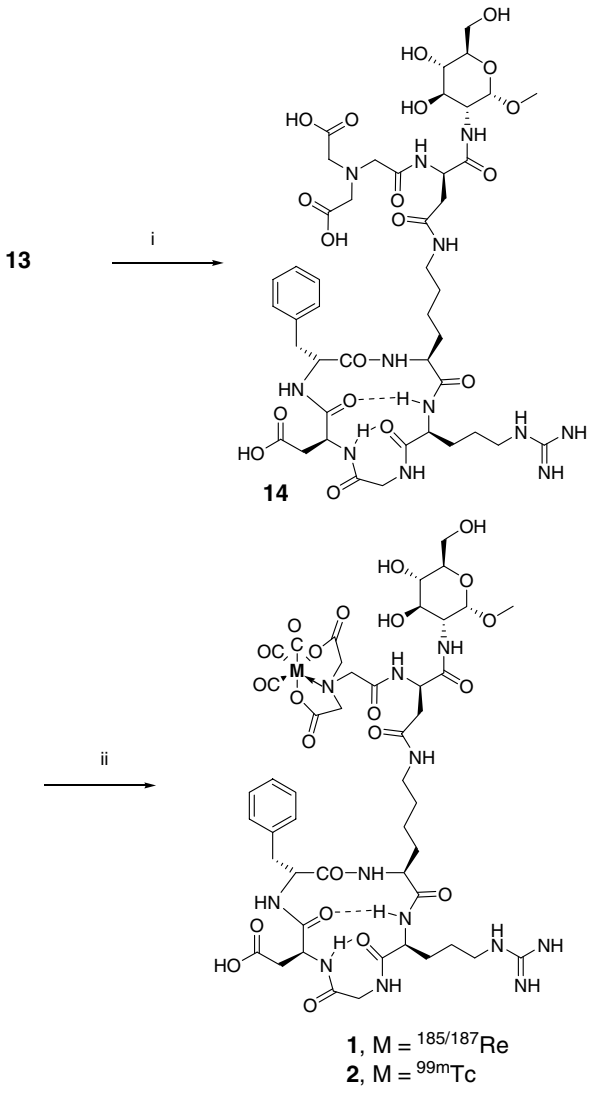
Competition binding assays were carried out on HUVE cells with cold authentic compounds or cRGDyV as shown in Figure 4. Inhibition studies demonstrated high specificity of [^{99m}Tc]**2** binding to $\alpha_v\beta_3$ integrin of HUVE cells. Cell bound levels decreased by 30.5% and 34.8% of control levels in the presence of 10 μM of cRGDyV and cold authentic compound **1**, respectively. If [^{99m}Tc]**2** remains to cell surface receptors, the results of this study suggest that [^{99m}Tc]**2** has high non-specific binding to the cells. In spite of this possibility, cell bound levels decreased around 30% of control levels in the presence of 10 μM of cRGDyV and cold authentic compound **1**, [^{99m}Tc]**2** re are quite high binding selectivity toward $\alpha_v\beta_3$ integrin.

2.5. Determination of IC_{50} value of glucosamino [^{99m}Tc]-D(RGDfK) binding to purified $\alpha_v\beta_3$ integrin

Competitive cell binding assays revealed a dose-dependent inhibition of [^{99m}Tc]**2** binding to immobilized $\alpha_v\beta_3$ integrin by non-radiolabeled cRGDyV. Binding in the presence of 1 μM cold RGD was markedly reduced to 8.4 ± 0.4% of control levels ($p < 0.00001$), indicating very low levels of non-specific binding. A sigmoid dose–response curve was obtained from the binding experiment from which high binding affinity with an apparent IC_{50} value of 1.5 nM was measured (Fig. 6).

3. Conclusion

Glucosamino-(Re)-D-c(RGDfK) (**1**) were synthesized using prosthetic groups linked to Asp-Lys in the RGD moiety with a glucosamino group followed by Re coordination, which should have little influence on selective binding to $\alpha_v\beta_3$ integrin. [^{99m}Tc]**2** was efficiently incorporated using tricarbonyl technetium-99m to NO_2 ligand attached to an amino terminal of an Asp residue in 90–92% radiochemical yield. [^{99m}Tc]**2** was shown to bind to human endothelial cells in $\alpha_v\beta_3$ integrin specific manners and with excellent binding affinities. Recently, in vivo work of [^{99m}Tc]**2** and its applications were pub-



Scheme 4. Reagents and conditions: (i) 10% Pd/C, H_2, AcOH/water, rt, 12 h; (ii) $(NEt_4)_2[ReCl_3(CO)_3]$, H_2O, 60 °C, 4 h, or $^{99m}Tc(CO)_3(H_2O)_3^+$, H_2O, 75 °C, 30 min.

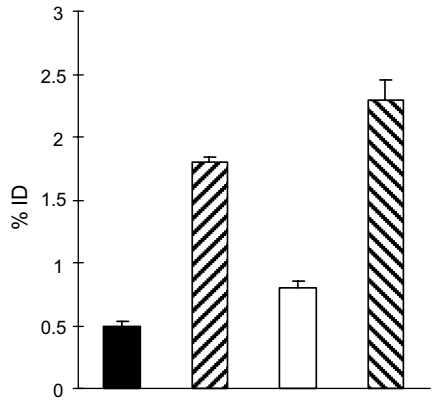


Figure 3. Endothelial cell binding of radiotracers. ECV304 cell binding levels are expressed as percent of added dose (%ID); closed bar: cRGD[^{125}I]yV was incubated for 60 min; right-handed striped bar: [^{99m}Tc]**2** was incubated for 60 min. HUVE cell binding levels are expressed as percent of added dose (%ID); open bar: cRGD[^{125}I]yV was incubated for 60 min; left-handed striped bar: [^{99m}Tc]**2** was incubated for 60 min.

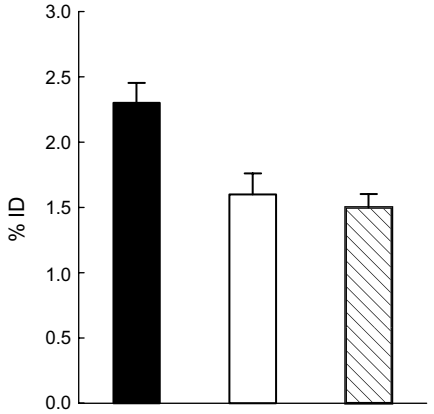


Figure 4. Binding competition of labeled RGD ([^{99m}Tc]**2**) and cRGDyV or **1**. HUVE cell binding levels are expressed as percent of added dose (%ID). [^{99m}Tc]**2** was incubated with the cells for 60 min in the presence or absence of 10 μM of either cold cRGDyV or authentic compound **1**; [^{99m}Tc]**2** uptake levels in the absence of competitors (closed bar); in the presence of cold cRGDyV (open bar); and in the presence of **1** (left-handed striped bar).

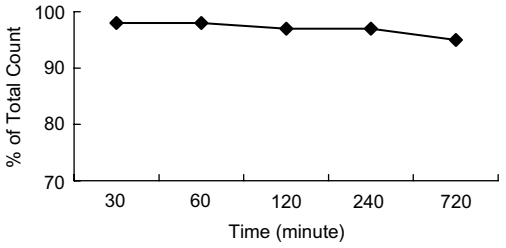


Figure 5. In vitro serum stability of [^{99m}Tc]**2**. The radiotracer (0.074–0.74 MBq) was incubated in human serum at 37 °C, and the percentage of intact radioactivity was analyzed by radio-TLC at 30, 60, 120, 240, and 720 min.

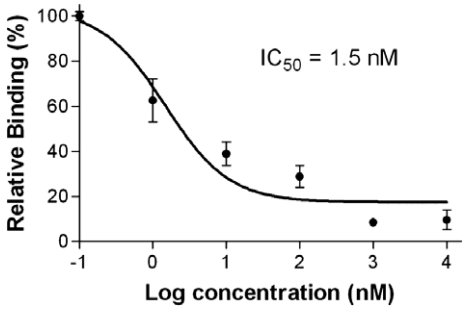


Figure 6. Specific endothelial binding characteristics of [^{99m}Tc]**2**. Competitive binding experiment results show concentration dependent inhibition of [^{99m}Tc]**2** binding to purified $\alpha_v\beta_3$ integrin by excess non-radiolabeled cRGDyV, and a sigmoidal dose–response curve with IC_{50} measurements. Data are expressed as means ± SE of triplicate samples.

lished elsewhere.[38] In addition, the synthesis and preparation of [^{99m}Tc]**2** are emphasized in this work. Thus, this radioprobe deserves further studies to evaluate their utilities for SPECT imaging of angiogenesis.

4. Experimental

4.1. Materials and general methods

Solvents and reagents were purchased from the following commercial sources: Sigma–Aldrich Company (Milwaukee, WI, USA) and Tokyo Chemical Industry (Tokyo, Japan). Fmoc amino acids were purchased from Advanced Chemtech (Louisville, KY, USA), NovaBiochem (Bad Soden, Germany), and Bead Tech (Seoul, Korea). The purified $\alpha_v\beta_3$ integrin was purchased from Chemicon (CC1019, Temecula, CA, USA). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini–400 (Palo Alto, CA, USA), and chemical shifts were reported in parts per million (ppm, δ units). Electron impact (EI) and chemical ionization (CI) mass spectra were obtained on a GC/MS QP5050A spectrometer (Shimadzu, Kyoto, Japan). Fast atom bombardment (FAB) mass spectra were obtained on a JMS 700 (Jeol Ltd, Tokyo, Japan). MALDI-TOF mass spectra were performed on Voyager-DE STR MALDI-TOF mass spectrometer (San Francisco, CA, USA). HPLC was carried out on a Thermo Separation Products System (Fremont, CA, USA) with a semipreparative column (Alltech Econosil silica gel, 10 μ, 10 × 250 mm) or an analytical column (YMC C18, 5 μ, 4.6 × 250 mm). The eluant was simultaneously monitored by a UV detector (254 nm) and a NaI(Tl) radioactivity detector. TLC was performed on Merck F_{254} silica plates and analyzed on a Bioscan radio-TLC scanner (Washington DC, USA). In vitro incubation was carried out at 37 °C using a block heater (Digi-Block Laboratory Device Inc., Holliston, MA, USA). $Na^{99m}TcO_4$ was eluted on a daily basis from ^{99}Mo/^{99m}Tc generators (DuPont Pharmaceuticals Co., Delaware, USA, and Daiichi Radioisotope Labs., Chiba, Japan). Radioactivity was measured in a dose calibrator (Perkin-Elmer, Wellesley, MA, USA).

The glucosamino derivatives **5** and **7** were prepared according to the literatures[30–34] and the cyclic pentapeptides; cyclic-Arg(Pbf)-Gly-Asp(O^{t}Bu)-D-Phe-Lys-NH_2 and cRGD[^{125}I]yV, were prepared as reported by Haubner et al.[15,16]

4.2. *N*-Bis(benzyloxycarbonylmethyl)glycine(O^{t}Bu) (3)

To a solution of glycine *tert*-butyl ester hydrochloride (1.05 g, 6.08 mmol) in CH_3CN (30 mL) were added benzyl bromoacetate (2.31 mL, 13.9 mmol) and *N*,*N*-diisopropylethylamine (DIEA, 5.30 mL, 30.4 mmol) at 0 °C. The reaction mixture was stirred for 5 h and allowed to attain room temperature. After saturated aqueous $NaHCO_3$ (10 mL) was poured, the crude product was extracted with CH_2Cl_2 (2× 25 mL). The organic layer was dried with anhydrous sodium sulfate and concentrated. The crude product was purified by flash column chromatography (EtOAc/hexane = 20:80) to give compound **3** (2.28 g, 88%) as a pale yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.33 (m, 10H, 2Ph), 5.14 (s, 4H, $PhCH_2$), 3.72 (s, 4H, CH_2), 3.58 (s, 3H, Gly-H$^\alpha$), 1.44 (s, 9H, O^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6, 169.9, 135.5, 128.5, 128.2, 81.2, 66.3, 55.7, 54.9, 28.0; MS (CI) *m*/*z* 428 (M+H$^+$), 400, 372 (100), 326, 280, 236, 91; HRMS (CI) calcd for $C_{24}H_{30}NO_6$ (M+H$^+$) 428.2076; found: 428.2073.

4.3. *N*-α-Bis(benzyloxycarbonylmethyl)glycine (4)

To a stirred solution of Boc-protected **3** (500 mg, 1.17 mmol) in CH_2Cl_2 (20 mL) at room temperature was added 10 mL of trifluoroacetic acid (TFA). The mixture was stirred at room temperature for 2 h and then the solvent was removed by azeotropy with toluene (10 mL) and dried in vacuo. The crude product was purified by flash column chromatography (MeOH/CH_2Cl_2 = 5:95) to give compound **4** (399 mg, 92%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.45 (br, 1H, Gly-OH), 7.38–7.33 (m, 10H, 2Ph), 5.17 (s, 4H, 2PhCH_2), 3.66 (s, 4H, CH_2), 3.57 (s, 3H, Gly-H$^\alpha$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 171.1, 134.9, 128.63, 128.59, 128.4, 67.2, 57.3, 56.1; MS (FAB) *m*/*z* 372 (M+H$^+$), 326, 280, 236, 91 (100), 57. HRMS (FAB) calcd for $C_{20}H_{22}NO_6$ (M+H$^+$) 372.1447; found: 372.1437.

4.4. Methyl 2-deoxy-3,4,6-tri-*O*-benzyl-2-(*N*-acetly,*N*-*tert*-butyloxycarbonylamino)-β-D-glucopyranoside (6)

A solution of **5** (700 mg, 1.39 mmol), DMAP (169 mg, 1.39 mmol), and Boc_2O (742 mg, 3.47 mmol) in dry THF (20 mL) was heated at 75 °C under N_2 gas. An additional amount of 385 mg (1.80 mmol) of Boc_2O and 55 mg (0.45 mmol) of DMAP was added every 6 h. After the mixture was left for 12 h at 75 °C, the reaction mixture was cooled at room temperature and 50 mL of CH_2Cl_2 was added. The organic layer was washed with 0.5 M H_2SO_4 (15 mL), water (15 mL), and saturated $NaHCO_3$ (20 mL), respectively. The organic layer was dried over anhydrous sodium sulfate and concentrated. The crude product was purified by flash column chromatography (EtOAc/hexane = 20:80) to give **6** (714 mg, 85%) as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36–7.15 (m, 15H, 3Ph), 4.85 (s, 2H, $PhCH_2$), 4.79 (d, J = 10.9 Hz, $PhCH_2$), 4.72 (d, J = 3.6 Hz, 1H), 4.47–4.62 (m, 2H), 4.53 (d, J = 11.2 Hz, 1H, $PhCH_2$), 4.52 (d, J = 12.4 Hz, 1H, $PhCH_2$), 3.85–3.82 (m, 1H, H-2), 3.78–3.66 (m, 3H, H-3, H-4, H-5), 3.32 (s, 3H, OCH_3), 2.22 (s, 3H, NAc), 1.50 (s, 9H, $3CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ172.28, 153.88, 139.24, 138.12, 137.83, 128.24, 128.22, 128.09, 127.68, 127.62, 127.51, 127.10, 126.90, 99.30, 83.48, 79.67, 79.47, 74.63, 73.98, 73.32, 70.51, 68.42, 54.82, 53.99, 28.26; MS (FAB) *m*/*z* 606 (M+H$^+$); HRMS calcd for $C_{35}H_{44}O_8N$ 606.3067; found: 606.3079.

4.5. Methyl 2-deoxy-3,4,6-tri-*O*-benzyl-2-*N*-[*N*-α-Fmoc-L-Asp(O^{t}Bu)]amino-α-D-glucopyranoside (8)

To a solution of Fmoc-Asp(O^{t}Bu)-OH (123 mg, 0.304 mmol), 1-hydroxybenzotriazole (HOBT, 49.4 mg, 0.365 mmol), and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, 117.3 mg, 0.365 mmol) in DMF (10 mL), the known compound **7** (141 mg, 0.304 mmol) and DIEA (69 μL, 0.396 mmol) in dry CH_2Cl_2 (3 mL) was added at room temperature under

N_2 gas. After the reaction mixture was stirred for 12 h, CH_2Cl_2 was evaporated. The crude product was extracted with EtOAc (2× 25 mL) and washed the saturated ammonium chloride (15 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated. The crude product was purified by flash column chromatography (EtOAc/hexane = 40:60) to give compound **8** (185 mg, 71%) as a white solid (mp 148.4–149 °C): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, J = 7.6 Hz, 2H, Fmoc), 7.56 (t, J = 7.0 Hz, 2H, Fmoc), 7.40 (t, J = 7.4 Hz, 2H, Fmoc), 7.36–7.11 (m, 17H, Fmoc, 3Ph), 6.69 (d, J = 9.6 Hz, 1H, NHCO), 6.02 (d, J = 8.0 Hz, 1H, NHCO), 4.76 (d, J = 10.8 Hz, 2H), 4.66–4.11 (m, 10H, $PhCH_2$, Asp-H$^{\alpha}$, Fmoc), 3.76–3.67 (m, 5H, H-3, 4, 5, H-6, H-6′), 3.33 (s, 3H, OCH_3), 2.84 (dd, J = 16.0, 4.4 Hz, 1H, Asp-H$^{\beta}$), 2.48 (dd, J = 16.0, 4.4 Hz, 1 H, Asp-H$^{\beta'}$), 1.42 (s, 9H, O^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 170.2, 155.9, 143.6, 141.2, 138.3, 138.0, 137.9, 128.3, 127.8, 127.74, 127.70, 127.6, 127.50, 127.46, 127.0, 125.0, 124.9, 120.0, 98.6, 81.7, 80.8, 77.9, 74.8, 73.3, 70.6, 68.4, 67.1, 55.1, 52.8, 51.0, 46.9, 37.3, 31.5, 27.9; MS (MALDI) m/z 879 (M+Na$^+$, 100), 656. HRMS (FAB) calcd for $C_{51}H_{57}N_2O_{10}$ (M+H$^+$) 857.4013; found: 857.4009.

4.6. Methyl 2-deoxy-3,4,6-tri-*O*-benzyl-2-*N*-{*N*-α-[*N*-α-bis(benzyloxycarbonylmethyl)-Gly]-L-Asp(O^{t}Bu)}amino-α-D-glucopyranoside (10)

Compound **10** was purified by flash column chromatography (EtOAc/hexane = 40:60) to give colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (d, J = 9.2 Hz, 1H, CONH), 7.38–7.20 (m, 23H, Ph), 7.14–7.11 (m, 2H, Ph), 6.91 (d, J = 9.6 Hz, 1H, CONH), 5.15 (s, 2H, CH_2, $PhCH_2$), 5.14 (s, 2H, CH_2, $PhCH_2$), 4.79 (d, J = 10.8 Hz, 1H, $PhCH_2$), 4.77–4.70 (m, 3H, $PhCH_2$, H-1), 4.62 (d, J = 10.8 Hz, 1H, $PhCH_2$), 4.52 (d, J = 12.0 Hz, 2H, $PhCH_2$), 4.33–4.27 (m, 1H, Asp-H$^{\alpha}$), 3.84–3.64 (m, 6H, H-2, H-3, 4, 5, H-6, H-6′), 3.61 (s, 2H), 3.59 (s, 2H), 3.41 (d, J = 2.0 Hz, 2H, Gly-H$^{\alpha}$), 3.30 (s, 3H, OCH_3), 2.79 (dd, J = 16.8, 5.8 Hz, 1H, Asp-H$^{\beta}$), 2.63 (dd, J = 16.8, 6.0 Hz, 1H, Asp-H$^{\beta'}$), 1.40 (s, 9H, O^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 170.7, 170.6, 170.5, 170.4, 138.4, 138.2, 138.0, 135.1, 128.7, 128.5, 128.31, 128.28, 127.79, 127.76, 127.70, 127.59, 127.57, 127.50, 98.7, 81.2, 80.7, 78.0, 74.9, 74.8, 73.4, 70.7, 68.9, 66.8, 58.3, 55.9, 55.1, 53.1, 49.2, 37.0, 28.0; MS (MALDI) m/z 1010 (M+Na$^+$, 100), 954. HRMS (FAB) calcd for $C_{56}H_{66}N_3O_{13}$ (M+H$^+$) 988.4596; found: 988.4618.

4.7. Methyl 2-deoxy-3,4,6-tri-*O*-benzyl-2-*N*-[NH_2-Asp(O^{t}Bu)]amino-α-D-glucopyranoside (9)

A solution of compound **8** (184 mg, 0.215 mmol) in 20% piperidine in DMF (5 mL) was stirred at room temperature. After 20 min, the crude product was extracted with EtOAc (2× 25 mL) and the organic layer was washed with the saturated ammonium chloride (20 mL) to remove DMF. After the organic layer was dried over anhydrous sodium sulfate and concentrated, the crude product was purified by flash column chromatography (EtOAc/hexane = 80:20) to give compound **9** (134 mg, 98%) as a white solid (mp 70.2–71.9 °C): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41–7.12 (m, 15H, 3Ph), 4.84 (d, J = 11.6 Hz, 1H, $PhCH_2$), 4.80 (d, J = 10.8 Hz, 1H, $PhCH_2$), 4.66–4.50 (m, 5H, $PhCH_2$, H-1), 4.27–4.22 (m, 1H, Asp-H$^{\alpha}$), 3.77–3.69 (m, 5H, H-3, H-4, H-5, H-6, H-6′), 3.47 (dd, J = 8.0, 3.4 Hz, 1H, H-2), 3.35 (s, 3H, OCH_3), 2.80 (dd, J = 16.0, 3.6 Hz, 1H, Asp-H$^{\beta}$), 2.43 (dd, J = 16.0, 9.2 Hz, 1H, Asp-H$^{\beta'}$), 1.54 (br, 2H, NH_2), 1.43 (s, 9H, O^{t}Bu); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.2, 171.1, 138.5, 137.93, 137.90, 128.29, 128.25, 127.9, 127.8, 127.6, 127.51, 127.49, 98.7, 80.94, 80.85, 78.3, 74.9, 73.3, 70.6, 68.4, 55.0, 52.3, 52.0, 40.3, 28.0; MS (MALDI) m/z 673 (M+K$^+$), 657 (M+Na$^+$, 100). HRMS (FAB) calcd for $C_{36}H_{47}N_2O_8$ (M+H$^+$) 635.3332; found: 635.3350.

4.8. Methyl 2-deoxy-3,4,6-tri-*O*-benzyl-2-*N*-{*N*-α-[*N*-α-bis(benzyloxycarbonylmethyl)-gly]-L-Asp-OH}amino-α-D-glucopyranoside (11)

To a solution of compound **10** (370 mg, 0.375 mmol) in CH_2Cl_2 (12 mL) was added trifluoroacetic acid (5 mL). The reaction mixture was stirred at room temperature for 2.5 h. The mixture was quenched with 15 mL of water and extracted with CH_2Cl_2 (2× 25 mL). The obtained organic layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The residue was purified with flash silica gel column chromatography (MeOH/CH_2Cl_2 = 7:93) to give compound **11** (345 mg, 99%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, J = 8.8 Hz, 1H, CONH), 7.39–7.21 (m, 23H, Ph), 7.16–7.14 (m, 2H, Ph), 7.02 (d, J = 9.2 Hz, 1H, CONH), 5.15 (s, 2H, CH_2, $PhCH_2$), 5.14 (s, 2H, CH_2, $PhCH_2$), 4.80 (d, J = 11.2 Hz, 1H, $PhCH_2$), 4.76–4.71 (m, 2H, $PhCH_2$, H-1), 4.67 (d, J = 12.0 Hz, 1H, $PhCH_2$), 4.62 (d, J = 12.0 Hz, 1H, $PhCH_2$), 4.52 (d, J = 12.0 Hz, 1H, $PhCH_2$), 4.50 (d, J = 11.2 Hz, 1H, $PhCH_2$), 4.31–4.25 (m, 1H, Asp-H$^{\alpha}$), 3.83 (m, 1H, H-2), 3.84–3.64 (m, 5H, H-3, 4, 5, H-6, H-6′), 3.59 (s, 2H), 3.57 (s, 2H), 3.42 (d, J = 8.0 Hz, 2H, Gly-H$^{\alpha}$), 3.28 (s, 3H, OCH_3), 2.84 (dd, J = 16.8, 5.6 Hz, 1H, Asp-H$^{\beta}$), 2.76 (dd, J = 16.8, 6.0 Hz, 1H, Asp-H$^{\beta'}$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.2, 171.5, 170.8, 170.6, 138.3, 138.1, 138.0, 135.1, 128.6 (×2), 128.5, 128.28 (×3), 128.26, 127.79, 127.73, 127.70, 127.6, 127.5, 98.6, 80.5, 78.1, 75.0, 74.8, 73.3, 70.7, 68.6, 66.9, 58.4, 56.0, 55.1, 53.2, 49.1, 35.6; MS (MALDI) m/z 954 (M+Na$^+$, 100). HRMS (FAB) calcd for $C_{52}H_{58}N_3O_{13}$ (M+H$^+$) 932.3970; found: 932.3961.

4.9. Tri-O-benzylglucosamino-[*N*-α-bis(benzyloxycarbonylmethyl)-Gly]-Asp-Arg(Pbf)-Gly-Asp(O^{t}Bu)-D-Phe-Lys (12)

To a solution of compound **11** (279 mg, 0.30 mmol), HOBT (48 mg, 0.36 mmol), and TBTU (115 mg, 0.36 mmol) in DMF (15 mL), was added a mixture of cyclic-R(Pbf)-G-D(O^{t}Bu)-f-K-NH_2 (273 mg, 0.30 mmol) and DIEA (67 μL, 0.39 mmol) in DMF (3 mL) at room temperature under N_2 gas. After the reaction mixture was stirred for 12 h, the solvent was removed under reduced pressure. Water (5 mL) was added to a small portion of mixture in DMF, the white solid appeared. This crude peptide was isolated by centrifugation and then the desired compound **12** was washed with 5% ethanol

in diethyl ether (3× 10 mL) and dried in vacuo. Analytical data were as follows: MS (MALDI) $m/z = 1849$ ($M+Na^+$, 100).

4.10. Tri-*O*-benzylglucosamino-[*N*-α-bis(benzyloxycarbonylmethyl)-Gly]-Asp-Arg-Gly-Asp-D-Phe-Lys (13)

To a solution of compound **12** (200 mg, 0.109 mmol) in a solution of trifluoroacetic acid/$HSCH_2CH_2SH/H_2O$ (5 mL, 95:2.5:2.5) was stirred at room temperature for 24 h. The reaction mixture was filtered with reaction solvent (2× 5 mL) and then the solvent was evaporated under reduced pressure. To get the solid, diethyl ether was added to the reaction mixture. The resulting solid was filtered and washed with diethyl ether. After the solid was dried under reduced pressure it gave compound **13** (88 mg, 53%) as a white solid; Analytical data were as follows: MS (MALDI) $m/z = 1517$ ($M+H^+$, 100), 1539 ($M+Na^+$).

4.11. Glucosamino-[*N*-α-bis(hydroxycarbonylmethyl)-Gly]-Asp-Arg-Gly-Asp-D-Phe-Lys (14)

To a solution of **13** (172 mg, 0.114 mmol) in a solution of AcOH (5 mL) and H_2O (5 mL) was added 269 mg (0.228 mmol) of the 10% palladium on charcoal under N_2 gas. After gas atmosphere was exchanged from N_2 to H_2 gas, the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was filtered through Celite layer and then the solvent was removed by azeotropy with toluene (5 mL) under reduced pressure. The crude mixture was purified by reverse phase HPLC. The desired compound **14** (84.5 mg, 83%) was obtained as a white solid. Analytical data were as follows: MS (MALDI) $m/z = 1068$ ($M+H^+$, 100), 1090 ($M+Na^+$).

4.12. Glucosamino (Re)-D-c(RGDfK) (1)

To a solution of compound **14** (10 mg, 9.37 μmol) in a solution of H_2O/MeOH (5 mL, 1:1) mixture was added 6.3 mg (10.3 μmol) of $(NEt_4)_2[ReCl_3(CO)_3]$. The reaction mixture was stirred at 65 °C for 1.5 h. The solution was removed under reduced pressure and then the product **1** was purified with a reverse pre-column (0–50% CH_3CN/0.1% TFA in H_2O, 214 nm, 40 min). Analytical data were as follows: MS (MALDI) $m/z = 1337$ ($M+H^+$). HRMS (FAB) calcd for $C_{47}H_{65}N_{12}O_{22}$ ^{187}Re ($M+H^+$) 1337.3972; found: 1337.3956.

4.13. Radiochemical synthesis of glucosamino [^{99m}Tc]-D-c(RGDfK) ([^{99m}Tc]2)

$[^{99m}Tc(H_2O)_3(CO)_3]^+$ was prepared according to the literature.[25–27] A solution of 200 μL of a mixture $[^{99m}Tc(H_2O)_3(CO)_3]^+$ (0.18–0.37 GBq) in saline was added to the precursor **14** (0.5 mg, 0.47 μmol) in H_2O (300 μL). The reaction mixture was stirred at 75 °C for 30 min and then cooled in an ice bath. The radiotracer was purified by HPLC at a flowrate of 1.5 mL/min using a solvent gradient (0–50% CH_3CN/0.1% TFA in H_2O for 30.1 min). The desired fraction of [^{99m}Tc]**2** was collected from HPLC and concentrated under reduced pressure. The residue was triturated with H_2O (200 μL) and loaded on a C-18 Sep-Pak cartridge, washed with H_2O (1 mL) and then methanol (1.5 mL). The product was dried under a stream of N_2 and diluted with PBS (pH 7.4). The overall radiochemical yield was 90–93%.

4.14. Cell culture

Human umbilical vein endothelial (HUVE) cells, known to express $\alpha_v\beta_3$ integrin,[39–42] were cultured in EGM (10 mL FBS + 0.2 mL hydrocortisone + 2 mL human recombinant epidermal growth factor + 0.5 mL VEGF + 0.5 mL R^3-IGF-1 + 0.5 mL ascorbic acid + 0.5 mL GA-1000 + 0.5 mL heparin) supplemented with 10% FBS, in a 37 °C incubator with 5% CO_2. Endothelium-derived ECV304 cells were cultured in M199 media supplemented with 10% FBS and antibiotic–antimycotics in a 37 °C incubator with 5% CO_2.

4.15. In vitro cell binding assays of labeled RGDs

For binding experiments, cells were harvested with 0.25% trypsin–1 mM EDTA, washed, and resuspended in D-PBS at a concentration of $2–3 \times 10^6$ cells per mL based on trypan blue dye exclusion assays. One hundred microliter of cell suspension in Eppendorf tubes was incubated with [^{99m}Tc]**2**, or cRGD[^{125}I]yV (0.037 MBq/tube) at 37 °C for 60 min. At the end of the incubation, cells were washed twice with 1 mL of D-PBS and underwent measurement of radioactivity on high-energy gamma counter along with standards.

4.16. Competitive cell binding experiments of labeled RGD, cRGDyV, and cold authentic compounds

Specificity of endothelial cell binding was evaluated by incubation of cells with [^{99m}Tc]**2** (0.037 MBq/tube) at 37 °C for 60 min as above, but in the presence of 10 μM of cRGDyV or cold authentic compound **1**. At the end of the incubation, cells were washed twice with D-PBS and measured for radioactive counts on a high-energy gamma counter along with standards.

4.17. In vitro stability studies

An aliquot (0.074–0.74 MBq) of [^{99m}Tc]**2** in 10% ethanol saline was mixed with 1 mL of human serum and incubated at 37 °C. The solution was analyzed at intervals of 30, 60, 120, 240, and 720 min by radio-TLC using *n*-butanol/water/acetic acid (3:1:1) as the developing solvents.[43]

4.18. Purified $\alpha_v\beta_3$ integrin binding assays

To assess affinities of the radioprobes, binding assays were performed on microtiter plates with $\alpha_v\beta_3$ integrin protein immobilized to the floor. Briefly, purified $\alpha_v\beta_3$ integrin diluted to 10 μg/mL in phosphate buffer (pH 5.8) was aliquoted to a 96-well microtiter plate in volumes of 50 μL/well (Medisorp, Nunc, Rochester, NY, USA). After overnight adhesion at 4 °C, the wells were blocked with blocking buffer containing 2.5 mg/mL casein for 30 min at 4 °C, then washed with PBS con-

taining 0.2 M NaCl and 0.05% Tween 20. For binding experiments, 40 μL of binding solution (PBS containing 0.1% bovine serum albumin) containing 370 kBq of [^{99m}Tc]**2** was applied to each well. Various concentrations of non-radiolabeled cRGDyV (0, 1, 10, 100, 1000, and 10,000 nM) were added to obtain competitive binding data. After incubation at room temperature for 3 h, each well was washed twice with PBS, separated from the plate, and measured for radioactivity on a high-energy gamma counter. The results were expressed as % activity relative to controls, as means ± SD of triplicate samples. Plotting of sigmoid competitive binding curves and the calculation of IC_{50} values were performed with GraphPad Prism version 3.00 for Windows (GraphPad Software).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.08.054.

References and notes

1. Ruoslahti, E.; Pierschbacher, M. D. *Science* **1987**, *238*, 491–497.
2. Cox, D.; Aoki, T.; Seki, J.; Motoyama, Y.; Yoshida, K. *Med. Res. Rev.* **1994**, *14*, 192–228.
3. Hynes, R. O. *Cell* **1992**, *69*, 11–25.
4. Brooks, P. C.; Montgomery, A. M.; Rosenfeld, M.; Reisfeld, R. A.; Hu, T.; Klier, G.; Cheresh, D. A. *Cell* **1994**, *79*, 1157–1164.
5. Brooks, P. C.; Stromblad, S.; Klemke, R.; Visscher, D.; Sarkar, F. H.; Cheresh, D. A. *J. Clin. Invest.* **1995**, *96*, 1815–1822.
6. Abumiya, T.; Lucero, J.; Heo, J. H.; Tagaya, M.; Koziol, J. A.; Copeland, B. R.; Del Zoppo, G. J. *J. Cereb. Blood Flow Metab.* **1999**, *19*, 1038–1050.
7. Lee, K.-H.; Jung, K.-H.; Song, S.-H.; Kim, D. H.; Lee, B. C.; Sung, H. J.; Han, Y.-M.; Choe, Y. S.; Chi, D. Y.; Kim, B.-T. *J. Nucl. Med.* **2005**, *46*, 472–478.
8. Brooks, P. C.; Clark, R. A.; Cheresh, D. A. *Science* **1994**, *264*, 569–571.
9. Ruoslahti, E.; Pierschbacher, M. D. *Cell* **1986**, *44*, 517–518.
10. Springer, T. A. *Nature* **1990**, *346*, 425–434.
11. D'Souza, S. E.; Ginsberg, M. H.; Matsueda, G. R.; Plow, E. F. *Nature* **1991**, *350*, 66–68.
12. Gurrath, M.; Muller, G.; Kessler, H.; Aumailley, M.; Timpl, R. *Eur. J. Biochem.* **1992**, *210*, 911–921.
13. Haubner, R.; Gratias, R.; Diefenbach, B.; Goodman, S. L.; Jonczyk, A.; Kessler, H. *J. Am. Chem. Soc.* **1996**, *118*, 7461–7472.
14. Haubner, R.; Finsinger, D.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1374–1389.
15. Haubner, R.; Wester, H. J.; Reuning, U.; Senekowitsch-Schmidtke, R.; Diefenbach, B.; Kessler, H.; Stocklin, G.; Schwaiger, M. *J. Nucl. Med.* **1999**, *40*, 1061–1071.
16. Haubner, R.; Wester, H. J.; Burkhart, F.; Senekowitsch-Schmidtke, R.; Weber, W.; Goodman, S. L.; Kessler, H.; Schwaiger, M. *J. Nucl. Med.* **2001**, *42*, 326–336.
17. Haubner, R.; Wester, H. J.; Weber, W. A.; Mang, C.; Ziegler, S. I.; Goodman, S. L.; Senekowitsch-Schmidtke, R.; Kessler, H.; Schwaiger, M. *Cancer Res.* **2001**, *61*, 1781–1785.
18. Haubner, R.; Kuhnast, B.; Mang, C.; Weber, W. A.; Kessler, H.; Wester, H.-J.; Schwaiger, M. *Bioconjug. Chem.* **2004**, *15*, 61–69.
19. Janssen, M. L.; Oyen, W. I.; Dijkgraaf, I.; Massuger, L. F.; Frielink, C.; Edwards, D. S.; Rajopadhye, M.; Boonstra, H.; Corstens, F. H.; Boerman, O. C. *Cancer Res.* **2002**, *62*, 6146–6151.
20. Liu, S.; Hsieh, W.-Y.; Kim, Y.-S.; Mohammed, S. I. *Bioconjug. Chem.* **2005**, *16*, 1580–1588.
21. Schottelius, M.; Rau, F.; Reubi, J. C.; Schwaiger, M.; Wester, H. J. *Bioconjug. Chem.* **2005**, *16*, 429–437.
22. Lohof, E.; Planker, E.; Mang, C.; Burkhart, F.; Dechantsreiter, M. A.; Haubner, R.; Wester, H.-J.; Schwaiger, M.; Hölzemann, G.; Goodman, S. L.; Kessler, H. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 2761–2764.
23. Schwochau, K. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 2258–2267.
24. Jurisson, S.; Berning, D.; Jia, W.; Ma, D. *Chem. Rev.* **1993**, *93*, 1137–1156.
25. Alberto, R.; Schibli, R.; Egli, A.; Schubiger, A. P.; Abram, U.; Kaden, T. A. *J. Am. Chem. Soc.* **1998**, *120*, 7987–7988.
26. Alberto, R.; Schibli, R.; Waibel, R.; Abram, U.; Schubiger, A. P. *Coord. Chem. Rev.* **1999**, *190–192*, 901–919.
27. Alberto, R.; Schibli, R.; Schubiger, A. P.; Abram, U.; Pietzsch, H.-P.; Johannsen, B. *J. Am. Chem. Soc.* **1999**, *121*, 6076–6077.
28. Schibli, R.; Netter, M.; Scapozza, L.; Birringer, M.; Schelling, P.; Dumas, C.; Schoch, J.; Schubiger, P. A. *J. Organomet. Chem.* **2003**, *668*, 67–74.
29. Schibli, R.; Schwarzbach, R.; Alberto, R.; Ortner, K.; Schmalle, H.; Dumas, C.; Egli, A.; Schubiger, P. A. *Bioconjug. Chem.* **2002**, *13*, 750–756.
30. Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503–1531.
31. Vasella, A.; Witzig, C.; Waldraff, C.; Uhlmann, P.; Briner, K.; Bernet, B.; Panza, L.; Husi, R. *Helv. Chim. Acta* **1993**, *76*, 2847–2875.
32. Fowler, P.; Bernet, B.; Vasella, A. *Helv. Chim. Acta* **1996**, *79*, 269–287.
33. Henry, C.; Joly, J.-P.; Chapleur, Y. *J. Carbohydr. Chem.* **1999**, *18*, 689–695.
34. Sherman, A. A.; Yudina, O. N.; Shashkov, A. S.; Menshov, V. M.; Nifant'ev, N. E. *Carbohydr. Res.* **2001**, *330*, 445–458.
35. Hawkes, M. J.; Ginsberg, A. P. *Inorg. Chem.* **1969**, *8*, 2189–2195.
36. Alberto, R.; Schibli, R.; Egli, A.; Schubiger, P. A.; Herrmann, W. A.; Artus, G.; Abram, U.; Kaden, T. A. *J. Organomet. Chem.* **1995**, *493*, 119–127.
37. Schibli, R.; La Bella, R.; Alberto, R.; Garcia-Garayoa, E.; Ortner, K.; Abram, U.; Schubiger, P. A. *Bioconjug. Chem.* **2000**, *11*, 345–351.
38. Jung, K.-H.; Lee, K.-H.; Paik, J.-Y.; Ko, B.-H.; Bae, J.-S.; Lee, B. C.; Sung, H. J.; Kim, D. H.; Choe, Y. S.; Chi, D. Y. *J. Nucl. Med.* **2006**, *47*, 2000–2007.
39. Pollman, M. J.; Naumovski, L.; Gibbons, G. H. *Circulation* **1999**, *99*, 2019–2026.

40. Kosugi, S.; Tomiyama, Y.; Honda, S.; Kashiwagi, H.; Shiraga, M.; Tadokoro, S.; Kiyoi, T.; Kurata, Y.; Matsuzawa, Y. *Thomb. Haemost.* **2001**, *85*, 36–41.
41. Su, Z.-F.; Liu, G.; Gupta, S.; Zhu, Z.; Rusckowski, M.; Hnatowich, D. J. *Bioconjug. Chem.* **2002**, *13*, 561–570.
42. Su, Z.-F.; He, J.; Rusckowski, M.; Hnatowich, D. J. *Nucl. Med. Biol.* **2003**, *30*, 141–149.
43. Lee, B. C.; Choe, Y. S.; Chi, D. Y.; Paik, J. Y.; Lee, K.-H.; Choi, Y.; Kim, B.-T. *Bioconjug. Chem.* **2004**, *15*, 121–127.