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Original Article

^{68}Ga-Labeling of RGD peptides and biodistribution

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Abstract: Several peptides comprising Arg-Gly-Asp (RGD) domain and macrocyclic chelator were labeled with ^{68}Ga for the imaging of angiogenesis. The analogues varied in peptide constitution, linker and chelator type. The labeling efficiency did not vary with the peptide constitution and linker type, but depended on the chelator type. Four of the compounds containing 2,2',2'',2'''-(1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrayl)tetraacetic acid (DOTA) chelator were labeled at 90 ± 5°C using conventional or microwave heating reaching 90% of ^{68}Ga incorporation after 5 and 2 min respectively, when the concentration of the precursor was 2.5 μM. The compound having 2,2',2''-(1,4,7-triazonane-1,4,7-triyl)triacetic acid (NOTA) as the chelator could be labeled at room temperature within 5 min using 2.5 μM peptide precursor. Two of the compounds contained a poly (ethylene glycol) (PEG) linker to the chelator. The biodistribution of the analogues was studied in male rats.

Keywords: ^{68}Ga, RGD, biodistribution

Introduction

The process by which new blood vessels are formed from pre-existing vessels is called angiogenesis [1]. Tumour and metastasis growth is believed to some extent be dependent on this phenomenon [2]. Radiolabeled peptides containing Arg-Gly-Asp (RGD) domain, having affinity towards the $\alpha_v\beta_3$ integrin receptors over expressed in angiogenesis, may be used for the diagnostic imaging, pretherapeutical dosimetry and therapy planning and monitoring. A number of publications reported on various radiolabeled RGD containing peptides with increased affinity to the $\alpha_v\beta_3$ integrin both *in vitro* and *in vivo* [3-10], allowing the distinction of overexpressing neoangiogenic endothelial cells from quiescent cells which do not express the integrins [11, 12].

Integrins are transmembraneous glycoproteins consisting of non-covalently bound α and β subunits which bind to extracellular matrix (ECM) components and are involved in regulation of intracellular signaling pathways [13]. The ligand is assumed to bind to the $\alpha_v\beta_3$ integrin by specific recognition of the RGD sequence [14, 15].

Poly(ethylene glycol) (PEG) is a linear polymer with hydrophilic properties [16]. PEG modifications are well-known to improve the therapeutical usefulness of proteins and peptides with regard to *in vivo* stability, pharmacokinetic, immunogenic and antigenic properties, and show little toxicity [16-18].

The positron-emitting radionuclide ^{68}Ga ($t_{1/2}$ = 68 min; 89% β^+-emission) is readily available from a long shelf-life ^{68}Ge/^{68}Ga-generator with the parent ^{68}Ge ($t_{½}$ = 270 days). The disintegration mode of the radionuclide results in high quality positron emission tomography (PET) images and allows accurate quantification.

In this study, RGD peptides with various modifications with regard to amino acid sequence and

using both DOTA and NOTA as chelators were labeled with ^{68}Ga. In addition, a peptide containing the scrambled sequence Gly-Asp-Phe (GDF) was labeled.

Materials and methods

Materials

DOTA-Glu-[cyclo (Arg-Gly-Asp-D-Phe-Lys)]$_2$ and cyclo [Arg-Gly-Asp-D-Phe-Lys]-PEG$_2$-NHCO-PEG$_2$-NH$_2$ were purchased from Peptides International, USA. Cys2-6; c[CH$_2$CO-Lys(DOTA)-Cys-Gly-Asp-Phe-Cys-Arg-Cys]-PEG$_3$-NHCOCH$_2$OCH$_2$CO-NH$_2$ (DOTA-AH-111170-02) DOTA-GDF-PEG and Cys2-6; c[CH$_2$CO-Lys(DOTA)-Cys-Arg-Gly-Asp-Cys-Phe-Cys]- PEG$_3$-NHCOCH$_2$OCH$_2$CO-NH$_2$, (DOTA-AH-110847-02) DOTA-RGD-PEG were obtained from Amersham Health (Dept. of Synthetic Chemistry, Amersham Health, Oslo, Norway). 2,2',2''-(10-{2-[(2,5-Dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid HPF$_6$·TFA (DOTA-NHS-ester) and 2,2',2''-[2-(4-isothiocyanatobenzyl)-1,4,7-triazonane-1,4,7-triyl]triacetic acid·3HCl (p-SCN-Bn-NOTA) were purchased from Macrocyclics (Dallas, USA). 2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES), trifluoroacetic acid (TFA), double-distilled hydrochloric acid (Riedel de Haën), sodium tetraborate decahydrate (Borax) and gallium (III) chloride were obtained from Sigma-Aldrich. Sodium hydroxide was obtained from Merck. The purchased chemicals were used without further purification. Millipore water (18 mΩ) from Purelab Maxima Elga system (Bucks, UK) was used throughout the synthesis.

Analytical methods

HPLC analyses of radiolabeled compounds were performed on a Beckman Nouveau HPLC system equipped with a Beckman 166 variable-wavelength UV detector and a Bioscan β$^+$-flow detector, using a gradient with water (A) and acetonitrile/water 70:30 (B), both containing 0.1% TFA, as mobile phase with UV detection at 220 nm. Program; 10% B for 2 min, then up to 70% in 8 min. A Grace Vydac C18 Protein and Peptide column (150 mm × 4.6 mm, 5μm) was used at a flow of 1 mL/min. For semipreparative HPLC a Grace Vydac C18 Protein and Peptide column (250 mm × 10 mm, 10μm) 300Å TP silica was used on a Gilson HPLC system at a flow of 5 mL/min. NMR spectra were recorded on a Varian Unity spectrometer at 400 MHz for ^{1}H, at 25°C in CD$_3$OD (solvent peak used as reference, ^{1}H 3.31 ppm). Chemical shifts (δ) are reported in ppm. Liquid chromatography mass spectrometry (LC-MS) analyses were performed using a Gilson HPLC and Finnigan AQA mass spectrometer, in ESI mode.

Microwave heating

Microwave heating was performed in a Smith-Creator™ monomodal cavity (Biotage AB, Uppsala, Sweden) at 2450 MHz. The temperature, pressure and microwave power were monitored and controlled. Smith Process Vial™ microwave vials of 200-1000 μL volume were used.

Preparation of ^{68}Ga

^{68}Ga ($t_{½}$ = 68 min, β$^+$ = 89% and EC = 11%) was available from a ^{68}Ge/^{68}Ga generator system (Cyclotron Co., Ltd, Obninsk, Russia) in which ^{68}Ge ($T_{½}$ = 270 d) was attached to a column of an inorganic matrix based on titanium dioxide. The ^{68}Ga was eluted with 6 mL of 0.1 M hydrochloric acid. The generator eluate was fractioned by discarding the first 0.8 mL and collecting the next 1-1.5 mL for the synthesis. The top fraction contained 70-75% of the total radioactivity.

General labeling procedure

HEPES (0.048 g, 0.20 mmol) was dissolved in 400 μL of ^{68}Ga eluate (in 0.1 M HCl (aq)) in a 2 mL Eppendorf tube. The pH was adjusted to 4.6-5 if necessary by adding 2 M NaOH (aq). Thereafter, an aqueous solution of RGD conjugates (1-5) (0.1-10 nmol), was added. The reaction was conducted at room temperature or by applying conventional heating at 90 ± 5°C for 5 min or microwave heating at 90 ± 5°C for 2 min.

Synthesis of reference compounds containing stable gallium isotopes (^{69,71}Ga)

HEPES (0.048 g, 0.20 mmol) was dissolved in ^{68}Ga eluate (200 μL) and the pH was adjusted to 4.6-5 by adding 2 M NaOH (aq). Thereafter, an aqueous solution of the respective RGD-precursor 1-5 (100 nmol) and ^{69,71}GaCl$_3$ (8.9 μL, 56 mM, 500 nmol) was added. The reaction mixture was heated at 90 ± 5°C for 5 min. ESI-MS: ^{69,71}Ga-1 *m/z* 886 [M+2H]$^{2+}$, 592 [M+3H]$^{3+}$; ^{69,71}Ga-2 *m/z* 1348 [M+H]$^+$, 674 [M+2H]$^{2+}$;

^{69,71}Ga-3 *m/z* 1414 $[M+H]^+$, 708 $[M+2H]^{2+}$, 477 $[M+3H]^{3+}$; ^{69,71}Ga-4 *m/z* 857 $[M+2H]^+$, 574 $[M+3H]^{3+}$; ^{69,71}Ga-5 *m/z* 857 $[M+2H]^{2+}$, 574 $[M+3H]^{3+}$.

Conjugation synthesis

Cyclo [Arg-Gly-Asp-D-Phe-Lys]-PEG$_2$-NHCO-PEG$_2$-NH-DOTA (2): Cyclo [Arg-Gly-Asp-D-Phe-Lys]-PEG$_2$-NHCO-PEG$_2$-NH$_2$ (0.002 g, 2.2 μmol) was dissolved in aqueous Borax (100 μL, 0.08 M, pH 9.3). The solution was cooled to 0°C and 2,2',2''-(10-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid HPF_6·TFA (0.023 g, 28 μmol) was added ending with a pH of 2. The pH was raised to 8 by adding 200 μL of a 1:1 v/v mixture of 1M NaOH (aq) and 0.08 M Borax (aq). The temperature of the reaction mixture was raised to room temperature and stirred for 20 h. Purification by semi-preparative HPLC (10-95% (B) over 8 min) gave the desired product (2) as a colourless oil (0.9mg, 31% yield). ^{1}H NMR (400 MHz, CD_3OD): δ = 7.32–7.26 (m, 2H), 7.24-7.18 (m, 3H), 4.11-4.04 (m, 1H), 3.95-3.88 (m, 1H), 3.81-3.77 (m, 8H), 3.67 (dd, 2H), 3.45-3.42 (m, 10H), 3.39 (t, 4H), 3.38-3.34 (m, 8H), 3.30 (m, 2H), 3.10 (t, 2H), 3.04 (t, 2H), 3.00-2.94 (m, 2H), 2.89-2.81 (m, 4H), 2.75-2.60 (m, 2H), 2.54 (m, 2H), 2.43-2.39 (m, 8H), 1.79 (qv, 2H), 1.75-1.33 (m, 6H), 0.88 (m, 2H). ESI-MS: *m/z* 1281 $[M+H]^+$, 641 $[M+2H]^{2+}$, 428 $[M+3H]^{3+}$. HPLC purity was >99%.

Cyclo [Arg-Gly-Asp-D-Phe-Lys]-PEG$_2$-NHCO-PEG$_2$-NH-NOTA (3): Cyclo [Arg-Gly-Asp-D-Phe-Lys]-PEG$_2$-NHCO-PEG$_2$-NH$_2$ (0.002 g, 2.2 μmol) was dissolved in aqueous Borax (100 μL, 0.08 M, pH 9.3). The solution was cooled to 0°C and 2,2',2''-[2-(4-isothiocyanatobenzyl)-1,4,7-triazonane-1,4,7-triyl]triacetic acid·3HCl (p-SCN-Bn-NOTA) (0.013 g, 22 μmol) was added ending with a pH of 2. The pH was raised to 8 by adding 200 μL of a 1:1 v/v mixture of 1M NaOH (aq) and Borax (aq). The temperature of the reaction mixture was raised to room temperature and stirred for 20 h. Purification by semi-preparative HPLC (10-90% (B) over 20 min) gave the desired product (3) as a colourless oil (0.0009g, 30% yield). ^{1}H NMR (400 MHz, CD_3OD): *δ* = 7.30–7.28 (AA'BB', 2H), 7.21-7.15 (m, 5H), 6.66 (AA'BB', 2H), 4.08-3.88 (m, 2H), 3.85-3.79 (m, 6H), 3.60 (dd, 2H), 3.42-3.39 (m, 10H), 3.38 (t, 4H), 3.35-3.32 (m, 8H), 3.28 (m, 2H), 3.07 (t, 2H), 3.01 (t, 2H), 3.00-2.81 (m, 2H), 2.73-2.57 (m, 2H), 2.51 (m, 2H), 2.40-2.28 (m, 9H), 1.74 (m, 2H), 1.71-1.30 (m, 6H), 0.85 (m, 2H). ESI-MS: *m/z* 1345 $[M+H]^+$, 674 $[M+2H]^{2+}$. HPLC purity was >99%.

Biodistribution study

Animals: All animals were handled in accordance with the guidelines by Swedish Animal Welfare Agency and animal experiments were conducted in accordance with approval of the veterinarian at Uppsala University. Animal studies were performed using healthy male Sprague Dawley (SPRD) rats weighting 347 g ± 17 (mean ± SD).

Organ distribution: In order to evaluate the uptake of the RGD analogues in normal tissues, uptake of radioactivity in a number of rat organs was investigated. Animals were divided into five groups (two animals per group). All the animals were anesthetized using Isoflurane mixed with O_2. Animals were injected intravenously into the tail vein with approximately 16 MBq (16.4 ± 2.0 MBq) per animal of one of the five ^{68}Ga-labeled compounds DOTA-$(RGD)_2$ (1), DOTA-PEG-RGD (2), NOTA-PEG-RGD (3) and DOTA-RGD-PEG (4), DOTA-GDF-PEG (5).

The animals were sacrificed 75 min post injection. Selected organs; blood, heart, lung, liver, spleen, adrenal, kidneys, intestine (with and without contents), muscle, bone, brain, pancreas, stomach, urinary bladder, bone marrow and tail were collected and weighed. The radioactivity was measured and corrected for decay in a gamma counter. Organs values were calculated as standard uptake value (SUV), as Equation 1:

$$SUV = \frac{ACT(Bq/g)}{DOSE(Bq)/BW(g)} \qquad \text{Equ.1}$$

where ACT is measured concentration of radioactivity expressed in Bq per gram tissue and corrected for physical decay, DOSE is administered amount of radioactivity (Bq), BW is body weight of the subject (g).

Results and discussion

Conjugation

The DOTA and NOTA conjugates 2-3 were prepared by reacting the 2,2',2''-(10-{2-[(2,5-dioxopyrrolidin-1-yl)oxy]-2-oxoethyl}-1,4,7,10-

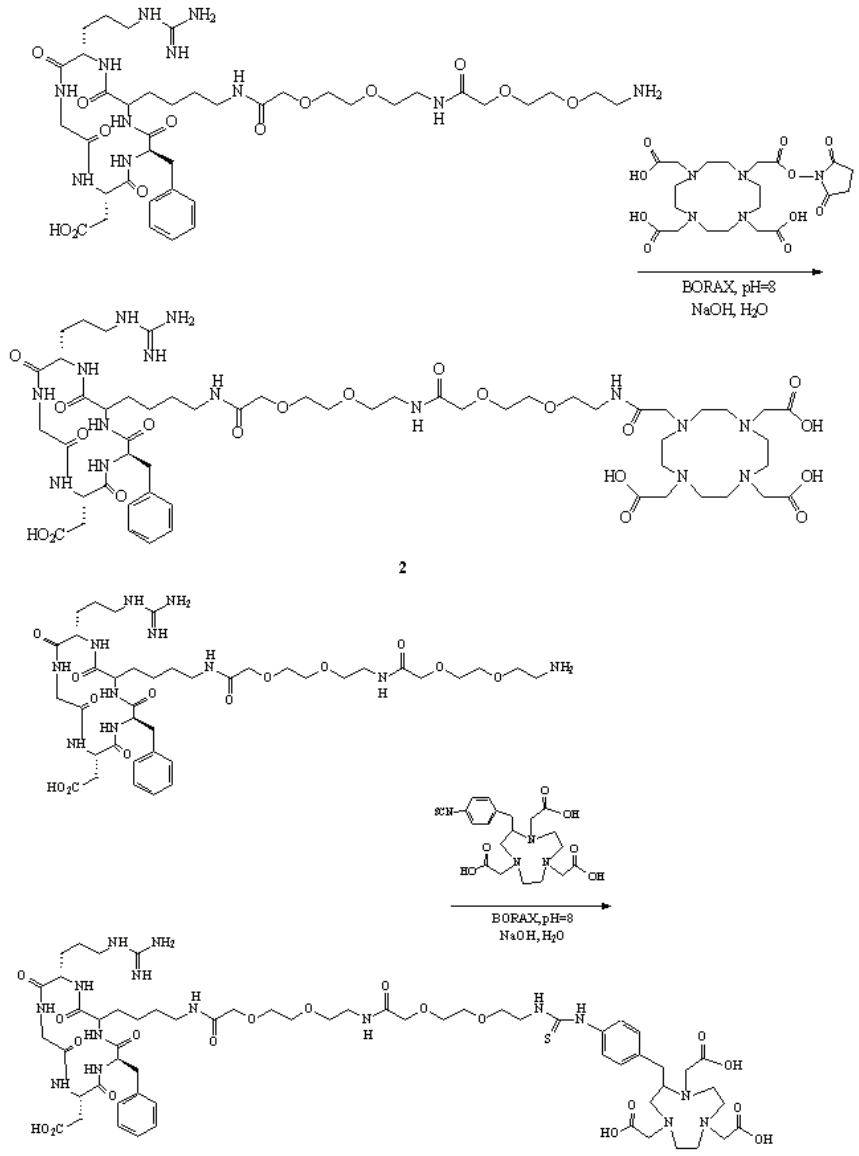

Figure 1. Synthesis of DOTA-PEG-RGD(2) and NOTA-PEG-RGD (3) from the amine.

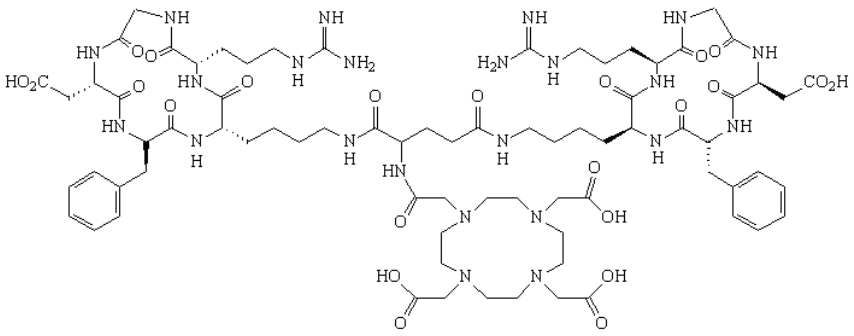

Figure 2. DOTA-$(RGD)_2$ (1).

tetraazacyclododecane-1,4,7-triyl)triacetic acid (DOTA-NHS-ester) or 2,2',2''-[2-(4-isothiocyanatobenzyl)-1,4,7-triazonane-1,4,7-triyl]triacetic acid 3HCl (p-SCN-Bn-NOTA) with the RGD-PEG-amine in water at pH 8 using a mixture of sodium tetraborate decahydrate (Borax) and sodium hydroxide as buffer (**Figure 1**). The yields of 2 and 3 were 30-31% after purification by semi-preparative HPLC.

Labeling

The size, amino acid constitution, PEG modifications as well as chelator type varied for the compounds under investigation. The influence of these variations on the labeling efficiency was studied. In compound 1 (**Figure 2**), which is commercially available, there are two RGD motifs present. This peptide has previously been labeled with ^{111}In, ^{90}Y, ^{99m}Tc [19], ^{64}Cu [20], and evaluated *in vivo* in nude mice. Compounds 2 and 3 (**Figure 1**) comprise a PEG linker between the peptide [21, 22] and the chelator. Compound 4 labeled with ^{68}Ga has been reported [23]. The retention times on the analytical HPLC column were in the range of 8.2-9.4 min for compounds 1-5, and they were increased by approximately 0.1 min when the gallium (III) ion was inserted.

The RGD conjugates were labeled with ^{68}Ga

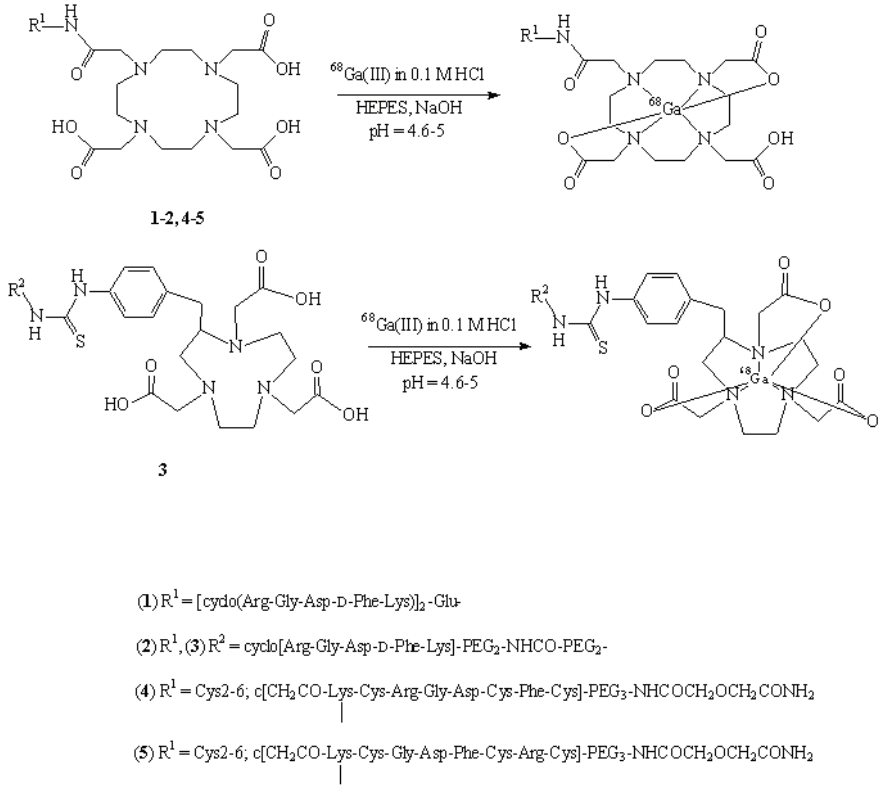

Figure 3. 68Ga-Labelling of RGD-DOTA and -NOTA analogues 1-5.

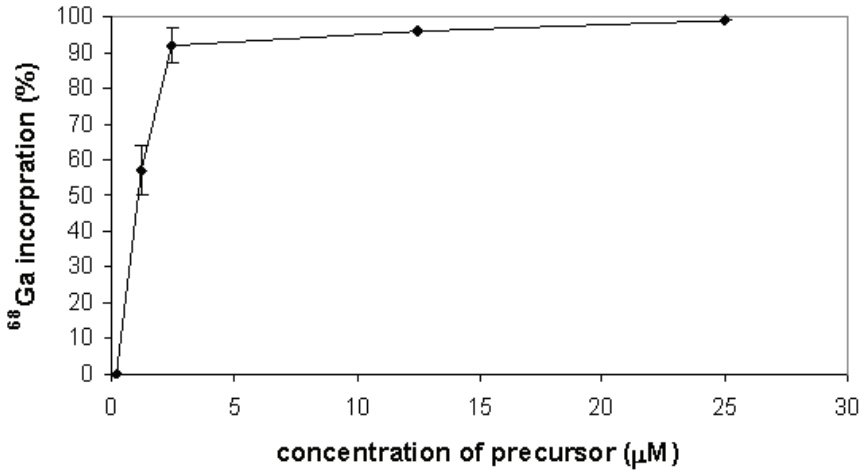

Figure 4. 68Ga incorporation versus concentration of precursor 1 at 90 ± 5ºC for 5 min in heating block. Data are presented as mean ± SD (n = 3).

(III) cation using a hydrochloric acid solution of the radiometal eluted from the 68Ge/68Ga generator system and 2-[4-(2-hydroxyethyl)piperazin -1-yl]ethanesulfonic acid (HEPES) as a buffer, as shown in **Figure 3**. The labeling reaction was performed at pH 4.6-5.0 at room temperature (compound 3) or at 90 ± 5°C, provided by conventional or microwave heating mode (compounds 1 and 2, 4 and 5). The degree of incorporation was determined by HPLC. The incorporation of 68Ga (III) as a function of the concentration of compound 1 (0.25-25 μM) under conventional heating at 90 ± 5°C for 5 min was investigated, and is shown in **Figure 4**. Compounds 1 and 2, and 4 and 5 were labeled at 90 ± 5°C, provided by conventional heating reaching 90% incorporation within 5 min, when using 2.5 μM concentration of the precursor. The NOTA-conjugate (3) could be labeled at room temperature reaching 95% incorporation within 5 min using 2.5 μM precursor concentration. Similar incorporation yields were obtained when using microwave heating at 90 ± 5°C, however, reaction time was reduced to 2 min. The 68Ga-labeling synthesis including subsequent HPLC analysis was accomplished within 30 min.

In **Figure 5** the specific radioactivity as a function of the concentration of compound 1 is shown, if the labeling mixture was not purified. If a certain level of radiochemical purity was required the specific radioactivity had to be decreased due to time

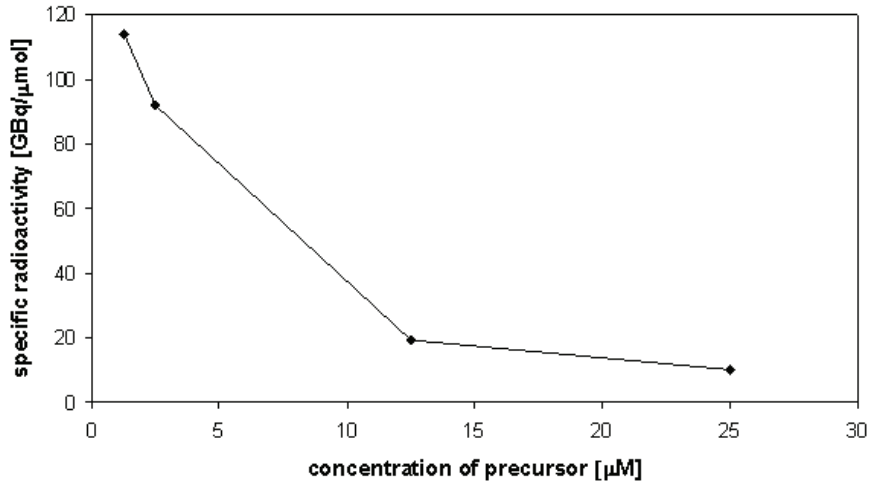

Figure 5. Specific radioactivity (end-of-synthesis) as a function of concentration of compound 1, 90 ± 5°C for 5 min in heating block, assuming the same amount of radioactivity at the end-of-synthesis (100 MBq).

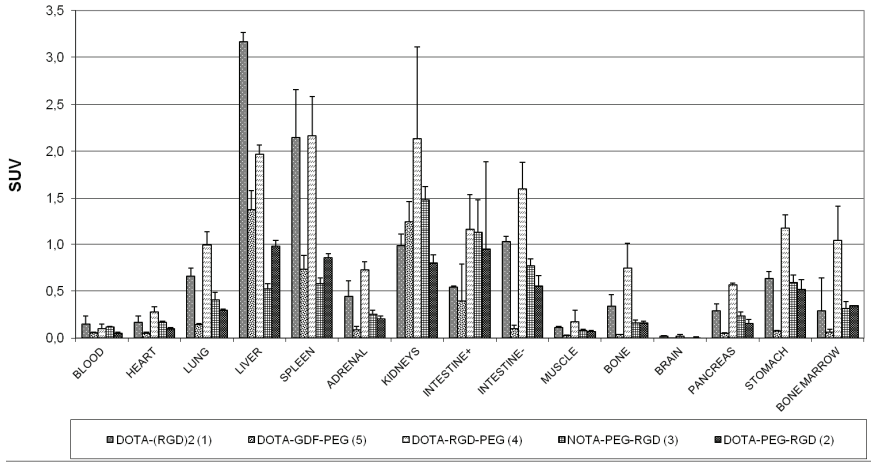

Figure 6. Biodistribution of ^{68}Ga-labelled DOTA-$(RGD)_2$ (1), DOTA-GDF-PEG (5), DOTA-RGD-PEG (4), NOTA-PEG-RGD (3) and DOTA-PEG-RGD (2) at 75 min post injection in male Sprague Dawley (SPRD) normal rats (n = 2). Average values were calculated and expressed as standard uptake value (SUV) (Mean ± SD) for different organs.

and radioactivity losses. As can be seen the highest specific radioactivity was obtained at the lowest concentration, 0.5 µM. If the labeling reaction could be performed in smaller volumes, also less amount of the precursor would be sufficient.

The advantage of using NOTA as the chelator, instead of DOTA, is that the labeling could be performed at room temperature. Moreover, gallium-NOTA complex [24, 25] having high symmetry octahedral coordination geometry has been shown higher thermodynamic stability as compared to gallium-DOTA complex where the chelator takes on a *cis*-pseudo-octahedral geometry when adding the gallium(III) ion [26].

Stable gallium isotopes (^{69,71}Ga) were introduced to the RGD conjugates using an aqueous solution of $GaCl_3$ at 90 ± 5°C for 5 min and the reaction conditions shown in **Figure 3**. The synthesis was conducted under the same conditions using a mixture of ^{68}Ga and ^{69,71}Ga cations. The identity of the compounds was confirmed by LC-ESI-MS. The stable gallium-labeled conjugates were added as authentic reference compounds in the LC analysis of labeling reactions in order to identify of the ^{68}Ga-labeled compounds.

Biodistribution

The results of the biodistribution studies of ^{68}Ga-labeled analogues, 1-5 are summarized in **Figure 6**. The individual organs were weighed and counted in a calibrated gamma counter. Standardized Uptake Value (SUV) was calculated for interesting organs.

All of the investigated compounds (1-5), labeled with ^{68}Ga, cleared rapidly from blood circulation. At 75 min post injection the radioactivity in blood was low for all analogues. The SUVs were 0.15 ± 0.1, 0.05 ± 0.01, 0.12 ± 0.01, 0.10 ± 0.05, and 0.06 ± 0.01 respectively for [^{68}Ga] DOTA-$(RGD)_2$ (1), [^{68}Ga]DOTA-PEG-RGD (2),

[^{68}Ga]NOTA-PEG-RGD (3), [^{68}Ga]DOTA-RGD-PEG (4), and [^{68}Ga]DOTA-GDF-PEG (5). In general, organs showing the highest uptakes were those expected to participate in excretion or metabolism, i.e. liver, kidneys, spleen and intestine, for all compounds. Moreover, the radioactivity in all organs was higher than that in blood, 75 min post injection, except for muscle, heart and brain. The former organs showed levels similar to those in blood, while the uptake in brain was only one fifth of that in blood, most certainly due to low blood brain barrier (BBB) penetration. The organ distribution was similar for [^{68}Ga] DOTA-PEG-RGD (2) and [^{68}Ga]NOTA-PEG-RGD (3), only differing in their chelating moiety, although slightly higher uptakes of radioactivity were found for compound 3 in all organs except in liver, which might indicate different elimination mechanisms.

Interestingly, uptake of [^{68}Ga]DOTA-GDF-PEG (5), used as a negative control, was lower in practically all organs, when compared with compounds 1-4, which contained the integrin binding domain, RGD. This behaviour of [^{68}Ga]DOTA-GDF-PEG was even more evident when compared with the uptake of [^{68}Ga]DOTA-RGD-PEG (4), which differed in structure only by the position of their amino acids.

In conclusion, five peptides, conjugated with NOTA or DOTA, of which four contained the RGD domain, were labeled at room temperature or by applying conventional or microwave heating at 90°C reaching >90% ^{68}Ga incorporation after 5 or 2 min respectively at 2.5 μM concentration. The impact of concentration and reaction temperature on specific radioactivity was studied. Biodistribution of the analogues was investigated 75 min post injection and it was found that organ distribution was similar for the analogues; with poor BBB penetration and rapid clearance from most other organs, except from those involved in elimination.

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Reference

[1] Folkman J. Tumor angiogenesis: therapeutic implications. New Engl J Med 1971; 285: 1182-1186.
[2] Folkman J. Role of angiogenesis in tumor growth and metastasis. Semin Oncol 2002; 29: 15-18.
[3] Janssen ML, Oyen WJ, Dijkgraaf I, Massuger LF, Frielink C, Edwards DS, Rajopadhye M, Boonstra H, Corstens FH, Boerman OC. Tumor targeting with radiolabeled αvβ3 integrin binding peptides in a nude mouse model. Cancer Res 2002; 62: 6146-6151.
[4] Kok RJ, Schraa AJ, Bos EJ, Moorlag HE, Asgeirsdottir SA, Everts M, Meijer DKF, Molema G. Preparation and Functional Evaluation of RGD-Modified Proteins as αvβ3 Integrin Directed Therapeutics. Bioconj Chem 2002; 13: 128-135.
[5] Garanger E, Boturyn D, Jin Z, Dumy P, Favrot M-C, Coll J-L. New Multifunctional Molecular Conjugate Vector for Targeting, Imaging, and Therapy of Tumors. Mol Ther 2005; 12: 1168-1175.
[6] Dijkgraaf I, Yim C-B, Franssen GM, Schuit RC, Luurtsema G, Liu S, Oyen WJG, Boerman OC. PET imaging of αvβ3 integrin expression in tumours with 68Ga-labelled mono-, di- and tetrameric RGD peptides. Eur J Nucl Med Mol Imaging 2011; 38: 128-137.
[7] Lee HY, Kang KW, Kim T-Y, Lee Y-S, Jeong JM, Han S-W, Paeng JC, Chung J-K, Lee MC, Lee DS. Angiogenesis imaging using 68Ga-RGD PET: preliminary report from Seoul National University Hospital. Curr Med Imaging Rev 2010; 6: 56-59.
[8] Liu Z, Niu G, Shi J, Liu S, Wang F, Liu S, Chen X. 68Ga-labeled cyclic RGD dimers with Gly3 and PEG4 linkers: promising agents for tumor integrin αvβ3 PET imaging. Eur J Nucl Med Mol imaging 2009; 36: 947-957.
[9] Li Z-B, Chen K, Chen X. 68Ga-labeled multimeric RGD peptides for microPET imaging of integrin αvβ3 expression. Eur J Nucl Med Mol Imaging 2008; 35: 1100-1108.
[10] Decristoforo C, Hernandez GI, Carlsen J, Rupprich M, Huisman M, Virgolini I, Wester H-J, Haubner R. 68Ga- and 111In-labelled DOTA-RGD peptides for imaging of alphavbeta3 integrin expression. Eur J Nucl Med Mol Imaging 2008; 35: 1507-1515.
[11] Brooks PC, Clark RA, Cheresh DA. Requirement of vascular integrin αvβ3 for angiogenesis. Science 1994; 264: 569-571.
[12] Horton MA. The αvβ3 integrin "vitronectin recep-

tor". Int J Biochem Cell Biol 1997; 29: 721-725.
[13] Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. Science 1987; 238: 491-497.
[14] Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992; 69: 11-25.
[15] Ruoslahti E. The RGD story: a personal account. Matrix Biology 2003; 22: 459-465.
[16] Davis FF, Abuchowski A, Van Es T, Palczuk NC, Chen R, Savoca K, Wieder K. Enzyme-polyethylene glycol adducts: modified enzymes with unique properties. Enzyme Engl 1978; 4: 169-173.
[17] Chen X, Park R, Shahinian AH, Bading JR, Conti PS. Pharmacokinetics and tumor retention of 125I-labeled RGD peptide are improved by PEGylation. Nucl Med Biol 2004; 31: 11-19.
[18] Roberts MJ, Bentley MD, Harris JM. Chemistry for peptide and protein PEGylation. Adv Drug Delivery Rev 2002; 54: 459-476.
[19] Janssen ML, Oyen WJ, Dijkgraaf I, Massuger LF, Frielink C, Edwards DS, Rajopadhye M, Boonstra H, Corstens FH, Boerman OC. Tumor targeting with radiolabeled αvβ3 integrin binding peptides in a nude mouse model. Cancer Res 2002; 62: 6146-6151.
[20] Chen X, Liu S, Hou Y, Tohme M, Park R, Bading JR, Conti PS. MicroPET imaging of breast cancer alphav-integrin expression with 64Cu-labeled dimeric RGD peptides. Mol Imaging Biol 2004; 6: 350-359.
[21] Dubey PK, Mishra V, Jain S, Mahor S, Vyas SP. Liposomes modified with cyclic RGD peptide for tumor targeting. J Drug Target 2004; 12: 257-264
[22] Schiffelers RM, Koning GA, ten Hagen TLM, Fens MHAM, Schraa AJ, Janssen APCA, Kok RJ, Molema G, Storm G. Anti-tumor efficacy of tumor vasculature-targeted liposomal doxorubicin. J Control Release 2003; 91: 115-122.
[23] Indrevoll B, Mørk Kindberg G, Solbakken M, Bjurgert E, Johansen JH, Karlsen H, Mendizabal M, Cuthbertson A. NC-100717: A versatile RGD peptide scaffold for angiogenesis imaging. Bioorg Med Chem Lett 2006; 16: 6190-6193.
[24] Broan CJ, Cox JPL, Craig AS, Kataky R, Parker D, Harrison A, Randall AM, Ferguson G. Structure and solution stability of indium and gallium complexes of 1,4,7-triazacyclononanetriacetate and of yttrium complexes of 1,4,7,10-tetraazacyclododecanetetraacetate and related ligands: kinetically stable complexes for use in imaging and radioimmunotherapy. X-ray molecular structure of the indium and gallium complexes of 1,4,7-triazacyclononane-1,4,7-triacetic acid. J Chem Soc Perkin Trans 1991; 21: 87-99.
[25] Craig AS, Parker D, Adams H, Bailey NA. Stability, gallium-71 NMR and crystal structure of a neutral gallium(III) complex of 1,4,7-triazacyclononanetriacetate: a potential radiopharmaceutical? J Chem Soc Chem Commun 1989; 23: 1793-1794.
[26] Heppeler A, Froidevaux S, Mäcke HR, Jermann E, Béhé M, Powell P, Hennig M. Radiometal-labelled macrocyclic chelator-derivatized somatostatin analogue with superb tumour-targeting properties and potential for receptor-mediated internal radiotherapy. Chem Eur J 1999; 5: 1974-1981.