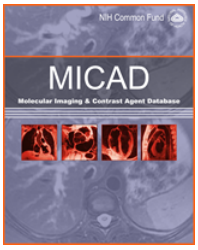
U.S. National Library of Medicine
National Center for Biotechnology Information

NLM Citation: Shan L. ^{111}In-Labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracetic acid-Glu{PEG$_4$-Glu[cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)]-cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)}-{PEG$_4$-Glu[cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)]-cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)} (PEG$_4$ = 15 amino-4,7,10,13-tetraoxapentadecanoic acid). 2012 Feb 23 [Updated 2012 Mar 22]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.
Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/



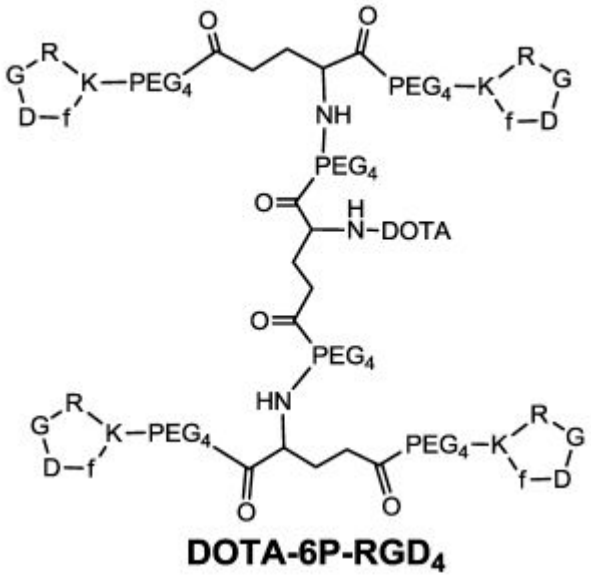
^{111}In-Labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracetic acid-Glu{PEG$_4$-Glu[cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)]-cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)}-{PEG$_4$-Glu[cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)]-cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)} (PEG$_4$ = 15 amino-4,7,10,13-tetraoxapentadecanoic acid)

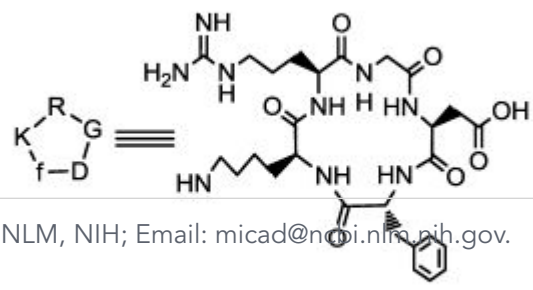
^{111}In(DOTA-6P-RGD$_4$)

Liang Shan, PhD[1]

Created: February 23, 2012; Updated: March 22, 2012.

Chemical name:	^{111}In-Labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracetic acid-Glu{PEG$_4$-Glu[cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)]-cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)}-{PEG$_4$-Glu[cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)]-cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)} (PEG$_4$ = 15 amino-4,7,10,13-tetraoxapentadecanoic acid)
Abbreviated name:	^{111}In(DOTA-6P-RGD$_4$)
Synonym:	^{111}In-DOTA-E{PEG$_4$-E[PEG$_4$-c(RGDfK)]$_2$}$_2$
Agent Category:	Peptides
Target:	Integrin alphavbeta3 ($\alpha_v\beta_3$)
Target Category:	Receptors
Method of detection:	Single-photon emission computed tomography (SPECT) or planar imaging
Source of signal / contrast:	^{111}In
Activation:	No





Author Affiliation: 1 National Center for Biotechnology Information, NLM, NIH; Email: micad@ncbi.nlm.nih.gov.

Table continued from previous page.

Studies:	• *In vitro* • Rodents	Structures of ^{111}In(DOTA-6P-RGD$_4$) by Shi et al. (1).

Background

[PubMed]

The ^{111}In-labeled 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetracetic acid (DOTA)-Glu{PEG$_4$-Glu[cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)]-cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)}-{PEG$_4$-Glu[cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)]-cyclo(Lys(PEG$_4$)-Arg-Gly-Asp-D-Phe)}, abbreviated as ^{111}In(DOTA-6P-RGD$_4$) or ^{111}In-DOTA-E{PEG$_4$-E[PEG$_4$-c(RGDfK)]$_2$}$_2$, was synthesized by Shi et al. as an agent for molecular imaging of tumor angiogenesis by targeting integrin alphavbeta3 ($\alpha_v\beta_3$) (1). Here, P = PEG$_4$ = 15 amino-4,710,13-tetraoxapentadecanoic acid = a linker between two c(RGDfK) motifs; c(RGDfK) = cyclo(Lys-Arg-Gly-Asp-D-Phe); RGD$_4$ = four motifs of c(RGDfK); and E = Glu.

Integrin $\alpha_v\beta_3$ is a receptor that is overexpressed on the activated endothelial cells of tumors (2). Because the integrin $\alpha_v\beta_3$ binds with extracellular matrix proteins (e.g., vitronectin, fibronectin) through the exposed Arg-Gly-Asp tripeptide sequence, RGD-containing peptides have been intensively studied in the past decade as a vector for imaging $\alpha_v\beta_3$ expression (3, 4). Although significant progress has been made, improving the binding affinity and pharmacokinetics of RGD peptides remains the major consideration in the design of agents, and various strategies have been developed for this improvement, such as the use of RGD multimers, the introduction of sugar amino acids or D-amino acids into the RGD peptides, and the conjugation of the RGD peptides with chelators or polyethylene glycol (PEG) chains (5, 6).

The concept of multimerization has been developed to address the question of multimeric binding by "poly-potent" ligands. Multimers are formed by bridging monomeric units through linker(s) (2, 7). The multimer effect has been demonstrated in different studies, showing that the binding affinity of RGD peptides increases in the order of monomer < dimer < tetramer < octamer. For example, the cyclo(-RGDfE-)-monomer, dimer, and tetramer containing heptaethylene glycol spacer units have been shown to exhibit $\alpha_v\beta_3$-binding affinities that increase by a factor of ten with each duplication of binding units (8). The integrin affinity of the DOTA-RGD octamer has been shown to be three-fold higher than that of the DOTA-RGD tetramer (7).

Recently, investigators from Purdue University suggested a concept of "bivalency" for the $\alpha_v\beta_3$-binding affinity of RGD-containing peptides (1, 6, 9). The main point of this concept is that the multimeric cyclic RGD peptides are likely bivalent rather than multivalent in binding to integrin $\alpha_v\beta_3$, and enough distance between two RGD motifs is the key for bivalency (1, 5, 10). For example, the dimeric peptide E[c(RGDfK)]$_2$ (RGD$_2$) is monovalent, whereas the dimeric peptides PEG$_4$-E[PEG$_4$-c(RGDfK)]$_2$ (3P-RGD$_2$) and G$_3$-E[G$_3$-c(RGDfK)]$_2$ (3G-RGD$_2$) (G = G$_3$ = Gly-Gly-Gly linker) are bivalent because of the increased distance between the two cyclic RGD motifs for simultaneous integrin $\alpha_v\beta_3$ binding in the latter two peptides (5). The cyclic RGD tetramers, such as DOTA-RGD$_4$ and DOTA-E{G$_3$-E[G$_3$-c(RGDfK)]$_2$}$_2$ (DOTA-6G-RGD$_4$), are also likely bivalent in binding to integrin $\alpha_v\beta_3$ even though they contain four identical RGD motifs (5, 10). Studies with three other tetramers (DOTA-2P-RGD$_4$, DOTA-2P4G-RGD$_4$, and DOTA-6P-RGD$_4$) have further shown that the three tetramers are not tetravalent, although the tetramers exhibited a higher binding affinity to $\alpha_v\beta_3$ than monomers and dimers, and the "locally enriched" RGD concentration has been considered to contribute to the higher binding affinity (1, 6). The linkers between RGD motifs may have a significant impact on the integrin $\alpha_v\beta_3$-targeting capability, biodistribution, excretion kinetics, and metabolic stability of cyclic RGD peptides (1, 11, 12).

Corresponding author.

This chapter summarizes the data obtained with ^{111}In(DOTA-6P-RGD$_4$). Other chapters summarize the data obtained with ^{111}In(DOTA-2P-RGD$_4$) and ^{111}In(DOTA-2P4G-RGD$_4$), respectively.

Related Resource Links:

The nucleotide and protein sequences of integrin $\alpha_v\beta_3$

Integrin $\alpha_v\beta_3$-related imaging agents in MICAD

Articles on integrin $\alpha_v\beta_3$ in Online Mendelian Inheritance in Man

Integrin $\alpha_v\beta_3$-related clinical trials in ClinicTrial.gov

Synthesis

[PubMed]

The RGD-containing peptides were all custom-made, including P-RGD, P2G-RGD$_2$, 3P-RGD$_2$, and 3P-RGK$_2$ (RGK = cyclo(Arg-Gly-Lys-D-Phe-Asp)). The tetramers were synthesized by the reaction of Boc-E(OSu)$_2$ and their corresponding dimers (1). Conjugation of the RGD peptides with DOTA-OSu resulted in their corresponding conjugates (DOTA-P-RGD, DOTA-P-RGD$_2$, DOTA-3P-RGD$_2$, DOTA-2P-RGD$_4$, DOTA-2P4G-RGD$_4$, and DOTA-6P-RGD$_4$, respectively). DOTA-6P-RGK$_4$ was prepared using a procedure identical to that for DOTA-6P-RGD$_4$. DOTA-6P-RGK$_4$ has the identical amino acids, but its sequence is scrambled to demonstrate the RGD-specificity of DOTA-6P-RGD$_4$. The identities for all RGD conjugates were confirmed, and their purities were all >95% before being used for ^{111}In labeling and determination of their integrin $\alpha_v\beta_3$ binding affinity. Table 1 lists the molecular weights and yields of each DOTA-conjugated peptide (1).

Table 1: The physicochemical characteristics of the RGD peptides.

Agents	DOTA-P-RGD	DOTA-P-RGD$_2$	DOTA-3P-RGD$_2$*	DOTA-2P-RGD$_4$	DOTA-2P4G-RGD$_4$	DOTA-6P-RGD$_4$	DOTA-6P-RGK$_4$
MW	1,237.58	1,951.5	2,447.35	3,626.4	4,315.4	4,622.7	4,622.7
Yield	~40%	~41%	~30%	~56%	~64%	~43%	~68%

*The data for DOTA-3P-RGD$_2$ were obtained from Shi et al. (5).

All ^{111}In-labeled peptides were prepared by reacting ^{111}InCl$_3$ with the respective DOTA conjugates in NH$_4$OAc buffer (100 mM, pH = 5.5). Radiolabeling was completed by heating the reaction mixture at 100°C for ~15 min. After purification, the radiochemical purity was >95% and the specific activity was >40 mCi/μmol (1.48 GBq/μmol) for all ^{111}In-labeled RGD peptides (1).

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The integrin binding affinity and specificity of the RGD peptides were assessed in U87MG human glioma whole cells, with ^{125}I-c(RGDyK) as the integrin-specific radioligand (1). The $\alpha_v\beta_3$ binding affinity of the peptides followed the order of DOTA-6P-RGD$_4$ ~ DOTA-2P4G-RGD$_4$ ~ DOTA-2P-RGD$_4$ > DOTA-P-RGD$_2$ > DOTA-P-RGD > RGD (IC_{50} = 49.9) (Table 1). The binding affinity of DOTA-3P-RGD$_2$ was significantly higher than that of DOTA-P-RGD and DOTA-P-RGD$_2$ ($P < 0.01$). However, the investigators found that the integrin $\alpha_v\beta_3$ binding affinities of DOTA-2P-RGD$_4$, DOTA-2P4G-RGD$_4$, and DOTA-6P-RGD$_4$ were only marginally higher than that of DOTA-3P-RGD$_2$, suggesting that they might share the same bivalency in binding to the integrin $\alpha_v\beta_3$.

Shi et al. also determined the water-octanol partition coefficients and stability of the ^{111}In-labeled peptides (1). The log *P* values are listed in Table 2. The ^{111}In-labeled peptides remained stable for >72 h after purification in the presence of 3 mM EDTA.

Table 2: The IC_{50} and log *P* values of the ^{111}In-labeled cyclic RGD peptides.

Peptides	DOTA-P-RGD	DOTA-P-RGD_2	DOTA-3P-RGD_2	DOTA-2P-RGD_4	DOTA-2P4G-RGD_4	DOTA-6P-RGD_4	DOTA-6P-RGK_4
IC_{50} (nM)	44.3	5.0	1.5	0.5	0.2	0.3	437
Log *P*	-3.48	-3.22	-4.20	-3.87	-3.93	-3.68	-3.61

IC_{50}: half-maximal inhibitory concentration.

Animal Studies

Rodents

[PubMed]

Biodistribution, imaging, and metabolic studies were performed in athymic nude mice bearing U87MG human glioma xenografts in both left and right upper flanks (1).

For the biodistribution studies, each mouse was administered ~3 μCi (~0.111 MBq) of the agent *via* tail vein injection (1). Mice were then euthanized at 0.5, 1, 4, 24, and 72 h after injection (*n* = 5 mice/time point). The organ uptake was calculated as the percentage of injected dose per gram of organ mass (% ID/g). ^{111}In(DOTA-6P-RGD_4) was excreted mainly *via* the renal route, with >75% of the injected radioactivity recovered from urine and <10% of the injected radioactivity from feces. ^{111}In(DOTA-6P-RGD_4) had a rapid blood clearance, with high tumor/blood ratios. In the tumor, the agent had a high initial uptake (10.5% ID/g at 0.5 h after injection), with a slow radioactivity washout (half maximal radioactivity = ~40 h). In the liver, ^{111}In(DOTA-6P-RGD_4) had a moderately low uptake and high tumor/liver ratios. However, ^{111}In(DOTA-6P-RGD_4) had significant uptake in the kidneys and intestine (Table 3).

Table 3: Selected biodistribution data of ^{111}In(DOTA-6P-RGD_4).

% ID/g	0.5 h	1 h	24 h	72 h
Blood	0.92 ± 0.06	0.29 ± 0.14	0.12 ± 0.02	0.06 ± 0.00
Kidney	10.68 ± 1.79	7.21 ± 0.79	4.27 ± 0.22	3.10 ± 0.38
Liver	4.09 ± 0.54	4.01 ± 0.41	1.50 ± 0.17	1.23 ± 0.27
U87MG	10.48 ± 1.83	11.08 ± 2.38	6.86 ± 1.87	3.04 ± 0.39
Tumor/Blood	11.32 ± 1.71	46.41 ± 25.60	56.45 ± 10.80	52.90 ± 4.95
Tumor/Kidney	0.99 ± 0.19	1.56 ± 0.41	1.60 ± 0.39	1.00 ± 0.21
Tumor/Liver	2.55 ± 0.21	2.79 ± 0.73	3.24 ± 1.02	2.60 ± 0.80
Tumor/Lungs	2.61 ± 0.33	3.13 ± 1.09	4.68 ± 1.72	4.70 ± 0.86
Tumor/Muscle	5.83 ± 0.80	10.90 ± 2.51	13.09 ± 2.85	13.47 ± 3.64

For the blocking experiment, each mouse was administered ~3 μCi (~0.111 MBq) ^{111}In(DOTA-6P-RGD_4) along with ~350 μg (~14 mg/kg) RGD_2 (*n* = 5 mice) (1). Animals were euthanized at 1 h after injection. Co-injection of excess RGD_2 significantly blocked the tumor uptake of ^{111}In(DOTA-6P-RGD_4) (0.7% ID/g with RGD_2*versus* 11.1% ID/g without RGD_2). The normal organ uptake of ^{111}In(DOTA-6P-RGD_4) was also blocked by the

presence of excess RGD$_2$. The uptake values of ^{111}In(DOTA-6P-RGD$_4$) without excess RGD$_2$ in the eyes, heart, intestine, liver, lungs, and spleen were 1.4, 0.8, 11.5, 4.0, 3.7, and 2.1% ID/g, respectively, while its uptake values with excess RGD$_2$ in the same organs were 0.1, 0.4, 0.5, 0.7, 1.2, and 0.5% ID/g, respectively (1).

To study the RGD specificity, comparative analysis was performed for tumor uptake values of ^{111}In(DOTA-6P-RGD$_4$) and control ^{111}In(DOTA-6P-RGK$_4$) at 60 min after injection (1). The control tetramer ^{111}In(DOTA-6P-RGK$_4$) had much lower tumor uptake values (0.8% ID/g) than those of ^{111}In(DOTA-6P-RGD$_4$) (11.1% ID/g) ($P < 0.01$). ^{111}In(DOTA-6P-RGK$_4$) also had significantly lower uptake values than those of ^{111}In(DOTA-6P-RGD$_4$) in the normal organs, such as intestine, kidneys, liver, lungs, and spleen ($P < 0.01$). These results indicate that replacing the RGD motifs with RGK will result in a dramatic uptake decrease in the tumor and $\alpha_v\beta_3$-positive organs, particularly the intestine, liver, lungs, and spleen.

The imaging studies were performed after tail vein administration of ~100 μCi (3.7 MBq) ^{111}In(DOTA-6P-RGD$_4$) ($n = 3$ mice) (1). The whole-body images were acquired at 0.5, 1, 4, 24, and 72 h after injection. The tumors were clearly visualized with excellent tumor/background contrast as early as 1 h after injection. A long tumor retention time was observed, which was consistent with the biodistribution results. The time to half-maximal radioactivity in tumors was >30 h for ^{111}In(DOTA-2P-RGD$_4$), ^{111}In(DOTA-2P4G-RGD$_4$), and ^{111}In(DOTA-6P-RGD$_4$) (1).

For the metabolism studies, each mouse was given ~100 μCi (3.7 MBq) ^{111}In(DOTA-6P-RGD$_4$) *via* tail vein injection (1). The urine samples were collected at 30 min and 120 min after injection, and the feces samples were collected at 120 min after injection. The percentage of radioactivity recovery was >95% (by γ-counting) for both urine and feces. There was very limited radioactivity accumulation in the liver and kidneys. ^{111}In(DOTA-6P-RGD$_4$) maintained its integrity during its excretion *via* the renal route; however, there was a significant metabolism during its excretion *via* the hepatobiliary route, with only ~45% of intact ^{111}In(DOTA-6P-RGD$_4$) in the feces sample.

Shi et al. comparatively analyzed the biodistribution data for ^{111}In(DOTA-2P-RGD$_4$), ^{111}In(DOTA-2P4G-RGD$_4$), ^{111}In(DOTA-6P-RGD$_4$), ^{111}In(DOTA-6G-RGD$_4$), ^{111}In(DOTA-3P-RGD$_2$), ^{111}In(DOTA-P-RGD$_2$), and ^{111}In(DOTA-P-RGD) (1). Over the first 24 h, the tumor uptake difference between the tetramers and dimers was not significant ($P > 0.05$). The tumor uptake at 72 h (% ID/g) followed the general order of ^{111}In(DOTA-6P-RGD$_4$) (3.04) > ^{111}In(DOTA-2P-RGD$_4$) (2.87) ~ ^{111}In(DOTA-2P4G-RGD$_4$) (2.66) > ^{111}In(DOTA-3P-RGD$_2$) (2.18), which was similar to the trend observed with integrin $\alpha_v\beta_3$ binding affinity (IC_{50}, nM) of DOTA-6P-RGD$_4$ (0.3 ± 0.1) ~ DOTA-2P4G-RGD$_4$ (0.2 ± 0.1) ~ DOTA-2P-RGD$_4$ (0.5 ± 0.1) > DOTA-3P-RGD$_2$ (1.5 ± 0.2). Among the ^{111}In-labeled radiotracers, ^{111}In(DOTA-6G-RGD$_4$) had the highest uptake in the tumor and intestine at 72 h after injection. The half-life of ^{111}In(DOTA-6G-RGD$_4$) in the tumor was estimated to be 60 h. In contrast, ^{111}In(DOTA-3P-RGD$_2$) and ^{111}In(DOTA-2P4G-RGD$_4$) had low uptake in the intestine, kidneys, and liver over the 72-h period. As a result, ^{111}In(DOTA-3P-RGD$_2$) and ^{111}In(DOTA-2P4G-RGD$_4$) had tumor/kidney and tumor/liver ratios that were significantly better ($P < 0.05$) than those of ^{111}In(DOTA-6G-RGD$_4$) and ^{111}In(DOTA-6P-RGD$_4$) during that period of time. As expected, ^{111}In(DOTA-P-RGD) had the lowest tumor uptake (3.7% ID/g and 0.7% ID/g at 0.5 h and 72 h after injection, respectively). ^{111}In(DOTA-P-RGD$_2$) had a relatively high tumor uptake (6.1 at 0.5 h after injection), but it had a significant washout from the tumor, with uptake values being 4.9, 4.8, 3.1, and 1.6% ID/g at 1, 4, 24, and 72 h after injection, respectively. The tumor uptake values follow the trend of ^{111}In(DOTA-3P-RGD$_2$) > ^{111}In(DOTA-P-RGD$_2$) > ^{111}In(DOTA-P-RGD), which was consistent with the order of their $\alpha_v\beta_3$ binding affinities (IC_{50} value): DOTA-3P-RGD$_2$ < DOTA-P-RGD$_2$ < DOTA-P-RGD.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

References

1. Shi J.et al. *Evaluation of In-Labeled Cyclic RGD Peptides: Effects of Peptide and Linker Multiplicity on Their Tumor Uptake, Excretion Kinetics and Metabolic Stability.* . Theranostics. 2011;1:322–40. PubMed PMID: 21850213.
2. Dijkgraaf I., Beer A.J., Wester H.J. *Application of RGD-containing peptides as imaging probes for alphavbeta3 expression.* . Front Biosci. 2009;14:887–99. PubMed PMID: 19273106.
3. Gaertner, F.C., M. Schwaiger, and A.J. Beer, *Molecular imaging of avb3 expression in cancer patients.* Q J Nucl Med Mol Imaging, 2010
4. Lu, X. and R.F. Wang, *A Concise Review of Current Radiopharmaceuticals in Tumor Angiogenesis Imaging.* Curr Pharm Des, 2012
5. Shi J.et al. *Impact of bifunctional chelators on biological properties of 111In-labeled cyclic peptide RGD dimers.* . Amino Acids. 2011;41(5):1059–70. PubMed PMID: 20052508.
6. Liu S. *Radiolabeled cyclic RGD peptides as integrin alpha(v)beta(3)-targeted radiotracers: maximizing binding affinity via bivalency.* . Bioconjug Chem. 2009;20(12):2199–213. PubMed PMID: 19719118.
7. Li Z.B.et al. *(64)Cu-labeled tetrameric and octameric RGD peptides for small-animal PET of tumor alpha(v)beta(3) integrin expression.* . J Nucl Med. 2007;48(7):1162–71. PubMed PMID: 17574975.
8. Poethko T.et al. *Chemoselective pre-conjugate radiohalogenation of unpretected mono- and multimeric peptides via oxime formation.* . Radiochim Acta. 2004;92:317–328.
9. Chakraborty S.et al. *Evaluation of 111In-labeled cyclic RGD peptides: tetrameric not tetravalent.* . Bioconjug Chem. 2010;21(5):969–78. PubMed PMID: 20387808.
10. Shi J.et al. *Improving tumor uptake and pharmacokinetics of (64)Cu-labeled cyclic RGD peptide dimers with Gly(3) and PEG(4) linkers.* . Bioconjug Chem. 2009;20(4):750–9. PubMed PMID: 19320477.
11. Liu Z.et al. *(68)Ga-labeled cyclic RGD dimers with Gly3 and PEG4 linkers: promising agents for tumor integrin alphavbeta3 PET imaging.* . Eur J Nucl Med Mol Imaging. 2009;36(6):947–57. PubMed PMID: 19159928.
12. Shi J.et al. *Improving tumor uptake and excretion kinetics of 99mTc-labeled cyclic arginine-glycine-aspartic (RGD) dimers with triglycine linkers.* . J Med Chem. 2008;51(24):7980–90. PubMed PMID: 19049428.