# $^{64}$Cu-1,4,7,10-Tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid-E{E[c(RGDyK)]$_2$}$_2$

## $^{64}$Cu-DOTA-E{E[c(RGDyK)]$_2$}$_2$

The MICAD Research Team

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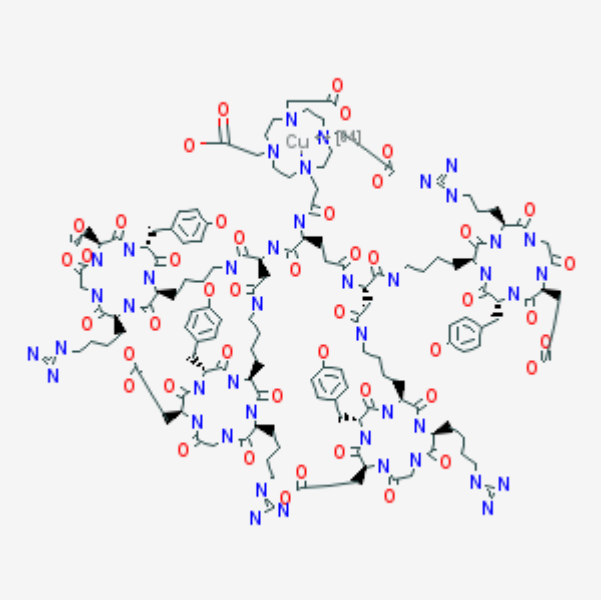
| **Chemical name:** | $^{64}$Cu-1,4,7,10-Tetraazacyclododecane-*N*,*N*',*N*'',*N*'''-tetraacetic acid- E{E[c(RGDyK)]$_2$}$_2$ |
| **Abbreviated name:** | $^{64}$Cu-DOTA-E{E[c(RGDyK)]$_2$}$_2$, $^{64}$Cu-tetrameric RGD D-Tyr analog |
| **Synonym:** | $^{64}$Cu-Tetrameric RGD peptide |
| **Agent Category:** | Peptide |
| **Target:** | Integrin $\alpha_v\beta_3$ |
| **Target Category:** | Receptor binding |
| **Method of detection:** | Positron Emission Tomography (PET) |
| **Source of signal:** | $^{64}$Cu |
| **Activation:** | No |
| **Studies:** | • *In vitro*<br>• Rodents |



Click on the above structure for additional information in PubChem.
Click on protein, nucleotide (RefSeq), and gene for more information about integrin $\alpha_v\beta_3$.

# Background

[PubMed]

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$^{64}$Cu-1,4,7,10-Tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid-E{E[c(RGDyK)]$_2$}$_2$ ($^{64}$Cu-DOTA-E{E[c(RGDyK)]$_2$}$_2$) is an integrin-targeted molecular imaging agent developed for positron emission tomography (PET) imaging of tumor vasculature, tumor angiogenesis, and osteoclasts (1). $^{64}$Cu is a positron emitter with a half-life ($t_{½}$) of 12.7 h.

Cellular survival, invasion, and migration control embryonic development, angiogenesis, tumor metastasis, and other physiological processes (2, 3). Among the molecules that regulate angiogenesis are integrins, which comprise a superfamily of cell-adhesion proteins that form heterodimeric receptors for extracellular matrix (ECM) molecules (4, 5). These transmembrane glycoproteins consist of two noncovalently associated subunits, α and β (18 α- and 8 β-subunits in mammals), which are assembled into at least 24 α/β pairs. Several integrins, such as integrin $\alpha_v\beta_3$, have affinity for the arginine-glycine-aspartic acid (RGD) tripeptide motif, which is found in many ECM proteins. Expression of integrin $\alpha_v\beta_3$ receptors on endothelial cells is stimulated by angiogenic factors and the environment. The integrin $\alpha_v\beta_3$ receptor is generally not found in normal tissue but it is strongly expressed in vessels with increased angiogenesis, such as tumor vasculature. It is significantly upregulated in certain types of tumor cells and in almost all tumor vasculature. Molecular imaging probes carrying the RGD motif that binds to the integrin $\alpha_v\beta_3$ can be used to image tumor vasculature and evaluate angiogenic response to tumor therapy (6, 7).

Various RGD peptides in both linear and cyclic forms have been developed for *in vivo* binding to integrin $\alpha_v\beta_3$ (8). It has been hypothesized that cyclic RGD peptides (RGDfK or RGDyK) may have a faster rate of receptor binding or a slower rate of dissociation from the integrin $\alpha_v\beta_3$ than linear single-RGD peptides (9). Chen et al (10). evaluated a cyclic RGD D-Tyr analog peptide [c(RGDyK)] labeled with $^{64}$Cu or $^{18}$F in nude mice bearing breast tumors. They used DOTA for c(RGDyK) conjugation with $^{64}$Cu; $^{64}$Cu-DOTA-c(RGDyK) showed prolonged tumor radioactivity retention but persistent liver radioactivity. Wu et al. (9) suggested that a multimeric RGD peptide with more than two repeating cyclic RGD units would further enhance the affinity of the receptor–ligand interactions through a polyvalency effect. They also suggested that the increase in molecular size might prolong circulation time and reduce tumor washout rate as well. Consequently, Wu et al. (9) developed the tetrameric RGD D-Phe analog peptide $^{64}$Cu-DOTA-E{E[c(RGDfK)]$_2$}$_2$ and showed that this PET radioligand appeared to have high integrin avidity and favorable biokinetics in nude mice bearing human gliomas. Li et al. (1) also prepared the tetrameric $^{64}$Cu-DOTA-E{E[c(RGDyK)]$_2$}$_2$ and octameric $^{64}$Cu-DOTA-E(E{E[c(RGDyK)]$_2$}$_2$)$_2$ for evaluation.

## Synthesis

[PubMed]

Li et al. (1) reported the synthesis of the tetrameric $^{64}$Cu-DOTA-E{E[c(RGDyK)]$_2$}$_2$. DOTA-E{E[c(RGDyK)]$_2$}$_2$ was synthesized through an active ester method by coupling Boc-Glu(OSu)-OSu with E[c(RGDyK)]$_2$ followed by deprotection. The monomeric cyclic

peptide c(RGDyK) was first prepared *via* solution cyclization of fully protected linear pentapeptide H-Gly-Asp(OtBu)-D-Tyr(OtBu)-Lys(Boc)-Arg(Pbf)-OH, followed by trifluoroacetic acid (TFA) deprotection in the presence of the free-radical scavenger triisopropylsilane (9, 11). The RGD dimer was then prepared by mixing c(RGDyK) with the Boc-protected, glutamic acid–activated ester Boc-Glu(OSu)$_2$ in triethylamine and stirring at room temperature overnight (11, 12). This yielded Boc-E[c(RGDyK)]$_2$. The Boc-group was then removed using anhydrous TFA. Similarly, the tetramer was prepared by mixing E[c(RGDyK)]$_2$ with Boc-E(OSu)$_2$ at room temperature (pH 8.5–9.0) overnight and removing the Boc-group by treatment with TFA. E{E[c(RGDyK)]$_2$}$_2$ was purified with high-performance liquid chromatography (HPLC) (9). DOTA-E{E[c(RGDyK)]$_2$}$_2$ was synthesized by first activating DOTA with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide and *N*-hydroxysulfonosuccinimide (pH 5.5) for 30 min to produce DOTA-OSSu. DOTA-OSSu was then cooled to 4°C and reacted with E{E[c(RGDyK)]$_2$}$_2$. The reaction mixture was adjusted to pH 8.5 and incubated overnight. The DOTA-peptide was purified with semi-preparative HPLC with a yield of 70%. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry showed a molecular weight of 3,199.0, which was in agreement with the calculated value of 3,198.4 ($C_{140}H_{207}N_{42}O_{45}$). $^{64}$Cu-DOTA-E{E[c(RGDyK)]$_2$}$_2$ was prepared by adding 37 MBq $^{64}$Cu-chloride/5 μg peptide (1 mCi/5 μg peptide) in sodium acetate to DOTA-E{E[c(RGDyK)]$_2$}$_2$ and incubating for 1 h at 50°C. The final radiolabeled peptide was purified by semi-preparative HPLC. The radiochemical yield was 80–90% (decay-corrected). The radiochemical purity was >98%, and the specific activity was 23 MBq/nmol (0.62 Ci/μmol).

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

Li et al. (1) conducted an *in vitro* cell integrin receptor-binding assay of unlabeled DOTA-E{E[c(RGDyK)]$_2$}$_2$, using $^{125}$I-echistatin as the integrin-specific radioligand and integrin $\alpha_v\beta_3$–positive human glioblastoma U87MG cells. The cell-binding assay demonstrated that DOTA had minimal effect on the receptor avidity. The 50% inhibitory concentration ($IC_{50}$) value for DOTA-E{E[c(RGDyK)]$_2$}$_2$ was (2.8 ± 0.4) × 10–$^8$ M ($n$ = 3). In comparison, the $IC_{50}$ value for E{E[c(RGDyK)]$_2$}$_2$ was (3.5 ± 0.3) × 10–$^8$ M. This tetramer also had ~three-fold higher affinity than the dimeric E[c(RGDyK)]$_2$. *In vitro* cell adhesion assays were also conducted to investigate the effect of E{E[c(RGDyK)]$_2$}$_2$ on U87MG cell adhesion (1). E{E[c(RGDyK)]$_2$}$_2$ had no effect on the cell adhesion ability in the presence of fibronectin, but the tetramer inhibited cell adhesion in a concentration-dependent manner in the presence of vitronectin. The calculated $IC_{50}$ value was (3.2 ± 0.9) × 10–$^7$ M ($n$ = 4). In comparison, the $IC_{50}$ value for the dimeric E[c(RGDyK)]$_2$ was (7.0 ± 1.0) × 10–$^7$ M.

# Animal Studies

## Rodents

[PubMed]

Biodistribution studies of $^{64}$Cu-DOTA-E{E[c(RGDyK)]$_2$}$_2$ were conducted in normal nude mice ($n$ = 3) (1). Each mouse received an i.v. dose of 0.74–1.11 MBq (20–30 μCi) $^{64}$Cu-DOTA-E{E[c(RGyK)]$_2$}$_2$ and was then euthanized 20 h after injection. The tumor radioactivity level in the kidney was 5.0 ± 0.7% injected dose per gram (% ID/g). The liver radioactivity level was ~2% ID/g (extrapolated from Figure 5A). The blood radioactivity level was ~0.1% ID/g, and the muscle radioactivity level was ~0.2% ID/g. Coinjection dose of 10 mg/kg c(RGDyK) significantly decreased the radioactivity in all tissues. The radioactivity level of the kidney was decreased to ~2.0% ID/g (extrapolated from Figure 5B). The liver radioactivity level was reduced to ~0.5% ID/g.

MicroPET imaging was conducted in nude mice bearing s.c. U87MG tumors (100–400 mm$^3$) and c-neu oncomice (integrin $\alpha_v\beta_3$-positive) that developed spontaneous mammary adenocarcinomas (1). Each mouse was injected with an i.v. dose of 9.3 MBq (0.25 mCi). $^{64}$Cu-DOTA-E{E[c(RGDyK)]$_2$}$_2$ clearly visualized the tumor with high tumor/background contrast up to 20 h after injection. Quantitative analyses of PET imaging data showed that the radioactivity levels in the U87MG tumors ($n$ = 3) were 10.3 ± 1.6% ID/g (0.5 h), 9.6 ± 1.4% ID/g (1 h), 8.6 ± 1.0% ID/g (2 h), 7.7 ± 1.6% ID/g (6 h), and 6.4 ± 0.7% ID/g (20 h). The liver radioactivity level was ~3% ID/g at 0.5 h and then decreased to ~2% ID/g at 20 h (extrapolated from Figure 4A). The kidney radioactivity level decreased from ~10% ID/g at 0.5 h to ~5% ID/g at 20 h, and the muscle radioactivity level decreased from ~1.5% ID/g at 0.5 h to ~0.5% ID/g at 20 h. In comparison, the tumor radioactivity appeared to be slightly higher than that of the D-Phe tetrameric analog $^{64}$Cu-DOTA-E{E[c(RGDfK)]$_2$}$_2$. The liver and kidney radioactivity levels of the D-Tyr and D-Phe analogs were similar. Coinjection with a dose of 10 mg/kg c(RGDyK) significantly decreased the radioactivity levels in the tumor, liver, kidney, and muscle at 2 h after injection. The tumor radioactivity level was reduced from ~8.8% ID/g to ~2.2% ID/g at 2 h after injection (extrapolated from Figure 5C). The kidney radioactivity level was reduced from ~5% ID/g to ~1.9% ID/g at 2 h after injection. In the c-neu oncomouse model of spontaneous mammary tumors, the tumor radioactivity levels were 4.4 ± 0.9% ID/g at 1 h and 3.6 ± 0.4% ID/g at 20 h. The liver and kidney radioactivity levels between 1 and 20 h after injection were ~2.5% ID/g and ~5% ID/g, respectively (extrapolated from Figure 4B). The muscle radioactivity level decreased from ~0.83% ID/g at 1 h to ~0.46% ID/g at 20 h.

Wu et al. (9) estimated the radiation-absorbed doses of $^{64}$Cu-DOTA-E{E[c(RGDfK)]$_2$}$_2$ in the adult human on the basis of the average biodistribution data in nude mice bearing U87MG tumors. The urinary bladder wall appeared to be the critical organ with 0.262 ± 0.0472 mGy/MBq (0.969 ± 0.175 rad/mCi). The total body dose was 0.00235 ± 0.000376 mGy/MBq (0.00869 ± 0.00139 rad/mCi). The estimated doses to the kidneys and liver

were 0.0296 ± 0.00184 mGy/MBq (0.110 ± 0.00684 rad/mCi) and 0.0243 ± 0.00156 mGy/MBq (0.0898 ± 0.00576 rad/mCi), respectively.

## Other Non-Primate Mammals

[PubMed]

No publication is currently available.

## Non-Human Primates

[PubMed]

No publication is currently available.

# Human Studies

[PubMed]

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