

# Synthesis of Gd-DOTA-Kinin Peptide Conjugates for Imaging Blood-Brain Barrier Opening in Malignant Glioma

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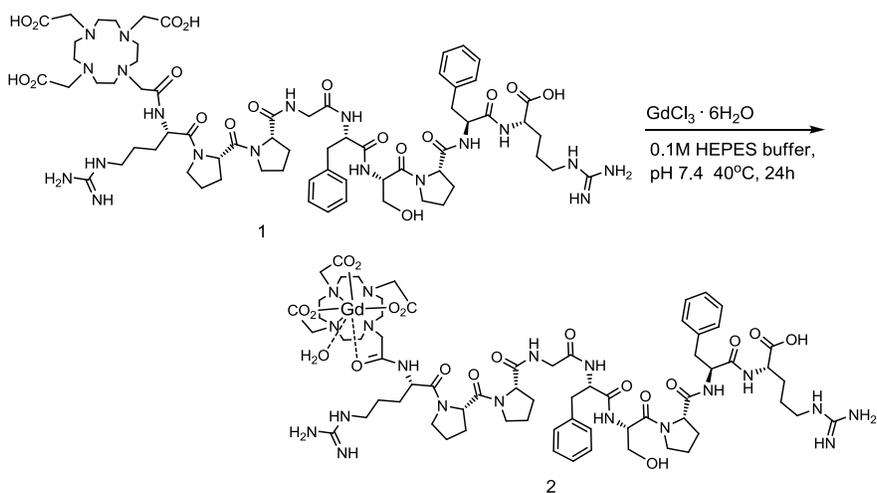
## Background

The most common primary brain tumor, malignant glioma (astrocytoma), usually recurs following surgery and adjuvant therapy, and is often refractory to systemic treatment with anti-glioma agents.<sup>1-3</sup> Kinin receptor agonists are vasoactive peptides known to increase glioma tumor barrier permeability by binding to over-expressed malignant kinin receptor subtypes B1 and B2. Agonistic activation of the kinin receptor leads to intra-tumoral nitric oxide release and selective temporary opening of the blood-brain tumor barrier (BBTB) in glioma to hydrophilic compounds up to six nanometers in diameter.<sup>4,5</sup> For this study, a series of Gadolinium-1, 4, 7, 10-tetraaza-1, 4, 7, 10-tetrakis(carboxymethyl) cyclododecane (Gd-DOTA)-Kinin peptide conjugates were synthesized. These conjugates were used to further investigate temporary kinin peptide-mediated opening of the BBTB using dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI).

## Chemistry

DOTA-Peptides (a-f) were chelated with gadolinium using a previously described method.<sup>6</sup> The solution was purified by centrifugation followed by high performance liquid chromatography (HPLC) to remove any unbound  $Gd^{3+}$  ions. The absence of free  $Gd^{3+}$  was confirmed by colorimetry using a xylenol orange indicator<sup>7</sup> and the products were stored at -80 °C.

## Scheme 1. Synthesis of Gd-DOTA-Kinin Peptides



## Experimental

**General.** DOTA-Kinin peptide conjugates were purchased from Peptides International, Inc (Louisville, Kentucky) and used as received. Gadolinium chloride was purchased from Sigma-Aldrich (Milwaukee, MI). Analytical and preparative HPLC analysis was performed on a Beckman Coulter System Gold using a Vydac C18 column (4.6 mm x 250 mm, 218TP54). Solvent A and B consisted of 0.05 % TFA in water and 0.05 % TFA in acetonitrile respectively. A linear gradient of 5 % B to 55 % B over 50 minutes with the flow rate of 1 mL/minute was used. Electrospray ionization mass spectrometry (ESI-MS) was performed on a LC/MSD TrapXCI Agilent Technologies instrument. The same conditions were used for preparative HPLC.

**Formation of DOTA-Kinin Peptide Gadolinium Complex (2)** To the DOTA-Kinin peptide (~10 mg) solution in 0.25 mL HEPES buffer (0.1 M, pH 7.4) was added 1.2 equivalents of  $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$ . The mixture was incubated at 40 °C for 24 h. Precipitates formed during the reaction were removed by centrifugation. The desired product was purified by HPLC. The product fraction was lyophilized to afford the complex as a white powder. The absence of free  $\text{Gd}^{3+}$  ions was confirmed with 0.1 % aqueous xlenol orange indicator at pH 5.5 and the products were stored at -80° C. The procedure stated above was followed for Gd chelation of the DOTA-Kinin peptides listed in Table 1. The products were characterized by ESI MS and HPLC. MS spectra showed a group of isotopically distinct peaks for the analogs.

**Table 1: Peptides and characterization**

Peptide sequence	Retention time of DOTA-peptide	Retention time of Gd-DOTA-peptide	Purity and yield	MS data: calculated : ESI MS found
DOTA-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH	21.5 min	21.5 min	97 %, 68.4 %	1603.6, 1601 (M)
DOTA-Met-Lys-Pro-Pro-Gly-Phe-Ser-Pro-Phe-OH	23.5 min	25 min	91 %, 82.1 %	1706.8, 1702 (M);
DOTA-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Tyr(Me)-Arg-OH	21.0 min	21.4 min	98 %, 80 %;	1633.7, 1630 (M);
DOTA-Sar-Arg-Pro-Pro-Gly-Phe-Ser-Pro-D-Phe-OH	23.0 min	25.2 min	82 %, 68.4 %;	1518.5, 1516 (M);
DOTA-Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH	22.0 min	22.4 min	93 %, 77.4 %;	1863.0, 1863 (M);
DOTA-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-OH	20.5 min	22.4 min	95 %, 73.6 %;	1731.8, 1731 (M).

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