# $(Gd\text{-}chelate)_2$-Phe-His-Cys-Pro(OH)-Tyr(2-Cl)-Asp-Leu-Cys-His-Ile-Leu-$(Gd\text{-}chelate)_2$

## 4Gdpeptide

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| **Chemical name:** | $(Gd\text{-}chelate)_2$-Phe-His-Cys-Pro(OH)-Tyr(2-Cl)-Asp-Leu-Cys-His-Ile-Leu-$(Gd\text{-}chelate)_2$ | |
| **Abbreviated name:** | 4GdPeptide | |
| **Synonym:** | EP-2104R (Gd-DOTA), EP-1873 (Gd-DTPA) | |
| **Agent category:** | Peptide | |
| **Target:** | Fibrin | |
| **Target category:** | Acceptor | |
| **Method of detection:** | Magnetic Resonance Imaging (MRI) | |
| **Source of signal/contrast:** | Gadolinium | |
| **Activation:** | No | |
| **Studies:** | • *In vitro*<br>• Non-primate non-rodent mammals | No structure is available in PubChem. |

# Background

[PubMed]

The acute formation of thrombus after atherosclerotic plaque rupture has been well recognized as the cause of unstable angina, myocardial infarction, transient ischemic attacks, and stroke (1, 2). Platelets and fibrin are the major components of all thrombi involved in the development and progression of atherosclerotic disease (3). MRI has shown promise in thrombus detection in both animals and humans (4), such as direct thrombus imaging based on the $T_1$-shortening properties of endogenous methemogobin

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in venous thrombi (5) and enhancing the contrast between the myocardium and intracardiac by conventional gadolinium chelates (6). However, thrombosis is a dynamic process in which the thrombus material of different ages forms a layered structure due to successive mural deposition (7). MR signal of thrombi is complicated by the presence of platelets, fibrin, and red blood cells in the clots (7). Accurate thrombus age definition and detection of old and organized thrombi remain difficult. Since fibrin is abundant in all types of thrombi, including arterial, venous, acute, and chronic, fibrin-targeted contrast agents can be used to differentiate between layers of fibrin deposits in all types of thrombi and in superimposed thrombosis associated with plaque (3). The low concentration of fibrin present in plasma minimizes spurious background imaging signal (3).

Fibrin is formed after thrombin cleavage of fibrinopeptide A from fibrinogen Aα-chains, followed by polymerization and cross-linkage to form thick fibrin bundles and complex branched clot network (8). 4GdPeptide is a gadolinium based contrast agent designed to bind to fibrin (7). 4GdPeptide comprises a small peptide of 11 amino acids and two gadolinium chelates such as gadolinium-diethylenetriaminepentaacetic acid (Gd-DTPA) or gadolinium-1,4,7,10-tetraazcyclododecane-*N,N',N'',N'''*-tetraacetate (Gd-DOTA) attached to each terminal (7, 9). Gd-DOTA has an *in vitro* stability five orders of magnitude greater than that of Gd-DTPA (10). This greater stability reduces the *in vivo* toxicity caused by the dissociation of free Gd(III) in the metabolic process (10). The small peptide contains one intramolecular disulfur bond, and binds to fibrin selectively and reversibly (9, 11). The binding to fibrin generates a field dependent paramagnetic enhancement effect (PRE) due to the increase of the effective rotation correlation time $\tau_R$ (12). No interactions and competitive binding with other proteins such as fibrinogen or collagen have been noted in both *in vitro* or *in vivo* (11). 4GdPeptide can penetrate through thrombi to bind fibrin in depth *via* passive diffusion; and distinguish between occlusive and non-occlusive arterial thrombi or between thrombi of different sizes and ages (11). Early-phase clinical studies (phase I and II) are currently testing the safety of 4GdPeptide in humans (11).

## Synthesis

[PubMed]

Bontar et al. reported a detailed synthesis of the cyclic disulfide precursor of EP-2104R/EP-1873 (7). Phe-His-Cys-Pro(OH)-Tyr(3-Cl)-Asp-Leu-Cys-His-Ile-Leu (FHCP(OH)Y(3-Cl)DLCHIL), was prepared with the use of a diaminotryl resin, Fmoc coupling strategy, and thallium trifluoroacetate cyclization. Conjugation of 4 back-bone substituted Gd-DTPA or Gd-DOTA was achieved with the use of standard amide coupling chemistry, acid deprotection and subsequent complexation with $GdCl_3$. There were four gadolinium chelates per peptide. The agent with chelates GD-DTPA was named as EP-1873 and that with chelates Gd-DOTA was EP-2104R. Both are commercially available (EPIX Medical Inc, MA).

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

[PubMed]

The relaxation efficacy of EP-1873, the agent with Gd-DTPA moieties, was measured at 1.4T and 37 °C (7). The value of $T_1$ relaxivity was 12 and 21 mM $Gd^{-1}$ $s^{-1}$ in buffer and fibrin, respectively. The binding of EP-1873 to fibrin was evaluated in Tris-buffered saline (7). $K_d$ was determined to be 3.5 ±0.15 μmol/L for rabbit fibrin and 2.2 ±0.3 μmol/L for human fibrin. The number of binding sites was 2.4 in rabbit fibrins and 1.8 in human fibrin. In comparison, the binding constant for soluble fibrinogen was >200 μmol/L. EP-2104R remained within the thrombus for 1.5 to 2 hr due to its high binding affinity for fibrin and apparent slow excretion kinetics relative to its blood clearance (11). No interactions and competitive binding with other proteins such as fibrinogen or collagen were reported for both *in vitro* and *in vivo* experiments.

## Animal Studies

### Rodents

[PubMed]

No publication is currently available.

### Other Non-Primate Mammals

[PubMed]

The specificity of EP-1873 binding for fibrin was examined in a rabbit carotid artery/jugular vein stasis model (7). After co-injection of 2 μmol/kg $^{153}$Gd-labeled EP-1873 and 2 μmol/kg $^{111}$In-labled-Gd-DTPA, the uptake of $^{153}$Gd-labeled EP-1873 (88±36 μmol/L) in carotid thrombus or jugular thrombi was significantly higher than that of $^{111}$In-labeled Gd-DTPA (2.5±0.5 μmol/L). At 60 min post injection, the blood level was found to be 22±8 μmol/L for $^{153}$Gd-labeled EP-1873 and 5.6±1.5 μmol/L for $^{111}$In-labeled Gd-DTPA. The mean residence time of EP-1873 in plasma was 47±10 min.

The use of EP-1873 to detect acute and subacute thrombosis was conducted in rabbits (7). Thrombus was induced on injured abdominal aortic walls and activated by pharmacological trigger after 8-week high-cholesterol diet. After bolus injection of 2 μmol/kg EP-1873, Images were collected at a 1.5 T imager. The signal intensity exhibited persistent increase in the acute thrombi. This led to a contrast to noise ratio (CNR) of 12±7 between the thrombus and blood one hour after injection and 24±10 twenty hours later, yielding an average signal to noise ratio (SNR) of 18±8 and 28±10 for the thrombus. In comparison, the average SNR for the arterial blood was 4.5±1.2 and 4.2±1.2. The subacute thrombus was examined 1-3 days after plaque rupture, for which the corresponding CNR was 8±2 one hour after injection and 19±7 twenty hours later. The detection of chronic thrombus was demonstrated in rabbits by using EP-2104R (11), the

agent with Gd-DOTA moieties. Arterial thrombosis was induced by carotid artery crush injury and blood stasis. The injured artery and the contralateral normal carotid artery were imaged at 5 μmol/kg dose shortly after thrombus induction. The images were also collected later as the thrombus advanced to different phases, including acute, subacute and chronic thrombus. EP-2104R can accurately detect occlusive and non-occlusive thrombi, regardless of their location, size, and organization stage.

The enhancement efficacy of EP-2104R was examined in the images of various thrombi in swine, including arterial thrombus (13), vein thrombus (14), pulmonary emboli (9, 15, 16), coronary thrombosis (15, 17) and atrial clots (18). In a coronary in-stent thrombosis model (13), all thrombi were observed after bolus injection of 60 μmol EP-2104R. The enhancement effect resulted in an average thrombus SNR and CNR of 11±2 and 9±2. Chemical analysis indicated that gadolinium concentration was 0.099 mM in a 38 mg thrombus and 0.147 mM in a 51 mg thrombus. In a cerebral sinus vein thrombosis model (14), acute thrombi formed in the superior sagittal sinus showed significant signal increase after bolus injection of 4 μmol/kg dose, allowing for discrimination between thrombus, blood pool and brain tissue. Gadolinium concentration in the thrombi ranged from 0.2 to 0.65 mM. The enhancement efficacy of EP-2104R in the clots made *ex vivo* was compared with that in the clots removed from patients (9, 17). In a pulmonary embolism (9), CNR was found to be 13±3 for the *ex vivo* engineered clots and 22±9 for the patient clots. Gadolinium concentration in the thrombi was determined to be 0.082±0.043 mM for the engineered clots and 0.247±0.044 mM for the patient clots respectively. Similar results were also found in a coronary arterial thrombosis in swine (17).

### Non-Human Primates

[PubMed]

No publication is currently available.

## Human Studies

[PubMed]

No details were currently available.

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