Electronic Supplementary Information (ESI)

Design, synthesis, and preliminary *ex vivo* and *in vivo* evaluation of cationic magnetic resonance contrast agent for rabbit articular cartilage imaging

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Experimental section

Materials and Equipments

All the reagents were used as received unless otherwise noted.

Analytical and preparative HPLC was conducted by use of COSMOSIL Cholester (4.6 x 150 mm and 20 x 250 mm, respectively, Nacalai Tesque Co.) as stationary phase and CH_3CN/0.1% aqueous solution of trifluoroacetic acid as mobile phase. ESI-MS was performed on Finnigan LCQ Advantage MAX (Thermo Fisher Scientific Inc). The magnetometric measurements were conducted on a MRI system (Agilent, UNITY INOVA^{TM}) with a 7-Tesla magnet (JASTEC) and a magnetic field gradient coil (Magnex).

The experiments using rabbits were approved by the Committee on Animal Care of Shiga University of Medical Science.

Synthesis of DOTA-Gd-G_2R_8

Fmoc-Arg_8(Pbf)-Nova Syn TGA resin (Fmoc: 9-fluorenylmethyloxycarbonyl, Arg: arginine, Pdf: 2,2,4,6,7-pentamethyldihydrobezofuran-5-sulfonyl) was prepared through conventional solid phase synthesis from Fmoc-Arg(Pbf)-Nova Syn TGA resin (0.23 mmol/g, Novabiochem) according to the literature.[1] After removal of the Fmoc protection with 20 % *N*, *N*-dimethylformamide (DMF) solution of piperidine, the resulting Arg_8(Pbf)-Nova Syn TGA resin was reacted with Fmoc-Gly-OH (Gly: glycine, 3.0 equiv, Novabiochem) in the presence of *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 3.0 equiv, Sigma Aldrich) and diisopropylethylamine (DIPEA, 6.5 equiv, Nacalai Tesque) in DMF for 3 h. This reaction was repeated again to give the Fmoc-Gly_2Arg_8(Pbf)-Nova Syn TGA resin. After removal of the Fmoc protection with 20 % DMF solution of piperidine, the resulting Gly_2Arg_8(Pbf)-Nova Syn TGA resin was reacted with 1,4,7,10-tetraazacyclododecane-1,4,7-

tris(*t*-butyl acetate)-10-aceitc acid (DOTA(tris-*t*-Bu ester), 2.5 equiv, Macrocyclics) in the presence of HATU (3.7 equiv) and DIPEA (6.5 equiv) in DMF for 12 h. The resulting DOTA(tris-*t*-Bu ester)-Gly_2Arg_8(Pbf)-Nova Syn TGA resin was treated with trifluoroacetic acid (TFA) solution including 80 % TFA (Nacalai Tesque), 1.4 % anisole (Wako Pure Chemical Industries), 1.4 % water, 0.6 % ethaneditiol (TCI), 0.6 % triisopropylsilane (TCI), and 16 % bromotrimethylsilane (TCI) to remove all the protection groups and the resin. After washing with methyl *t*-butyl ether followed by freeze-drying the solid, a aqueous solution of DOTA-Gly_2Arg_8 at 16 mM was reacted with $GdCl_3$ (1.3 equiv) at 80 °C for 12 h. A white solid was recovered under basic condition (pH = 11) through filtration and freeze-dried to give crude product of DOTA-Gd-G_2R_8, which was purified with HPLC and identified with ESI-MS (Fig. S1 and S2, respectively).

MR *ex vivo* imaging of articular cartilage with DOTA-Gd-G_2R_8

A femoral condyle from a rabbit was fixed in a cylinder (Fig. S4a) and immobilised in the coil (Fig. S4b). The medium in the cylinder was changed remotely through the inlet and outlet tubes with fixation of the sample at the same place in the sequence of the MR measurements. This equipment enabled the precise comparison at the same slice of the images in the time course of the MR measurement. The femoral condyle was measured firstly in PBS, secondly in a PBS solution of DOTA-Gd-G_2R_8 (1.0 mM), and lastly in PBS again. MR images at 18 slices were obtained by a spin echo sequence (repetition time (TR) = 792 ms and echo time (TE) = 19 ms).

Safranine *O* staining

Specimens were fixed in 20% formalin at 4 ° C for 48 hours, decalcified with K-CX (Falma, Tokyo, Japan) for 1 – 2 weeks, and then embedded in paraffin. Each specimen was cut sagittally into 3-μm-thick sections. After deparaffinization followed by dehydration, the sections were

immersed in Safranine *O* (Chroma Gesellschaft Schmidt & Co.) for 5 min and fast green (Wako Pure Chemical Industries, Ltd.) for 5 min.

MR *in vivo* imaging of articular cartilage with DOTA-Gd-G_2R_8

The knee joint of a rabbit was fixed in the coil and the contrast agent was injected remotely through the indwelling needle (Fig. S5). When 0.8 mL of the PBS solution (1.0 mM) of DOTA-Gd-G_2R_8 was injected into the knee joint, no significant change in the contrast was observed neither in AC nor in synovial fluid. Therefore, more amount (2.0 mL) of the same solution was injected additionally and the joint was monitored with MR until 5 h after the injection as shown in Fig. 5. MR images at 24 slices were obtained by a spin echo sequence (TR = 1100 ms, TE = 19 ms).

Reference

1. M. J. Allen, K. W. MacRenaris, P. N. Venkatasubramanian and T. J. Meade, *Chem. Biol.*, 2004, **11**, 301-307.

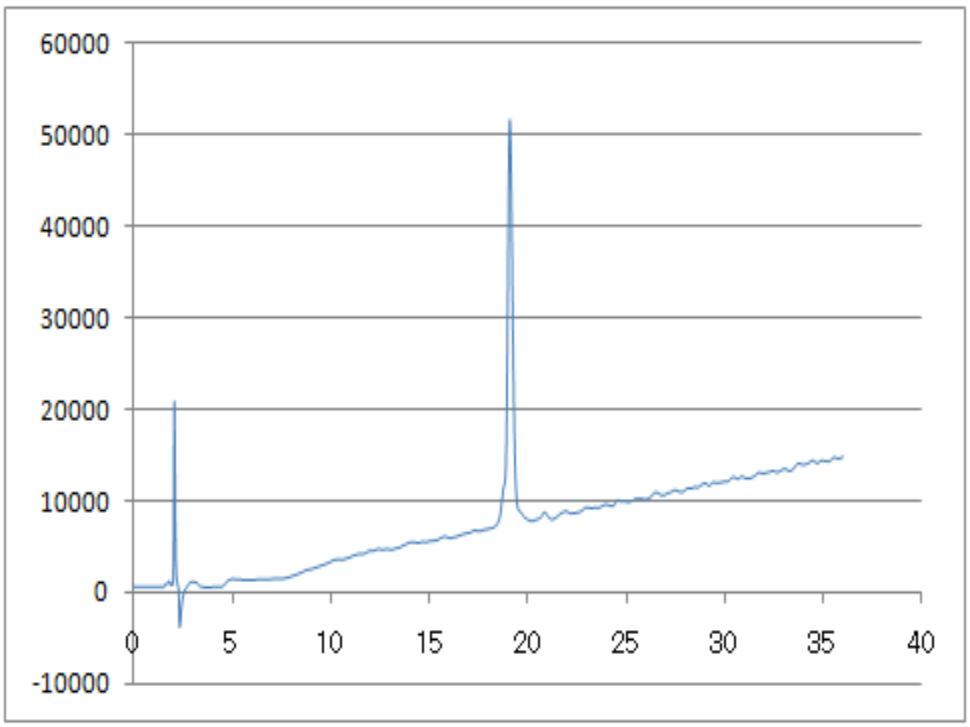

Fig. S1. Analytical HPLC chromatogram of DOTA-Gd-G_2R_8 after purification with preparative HPLC

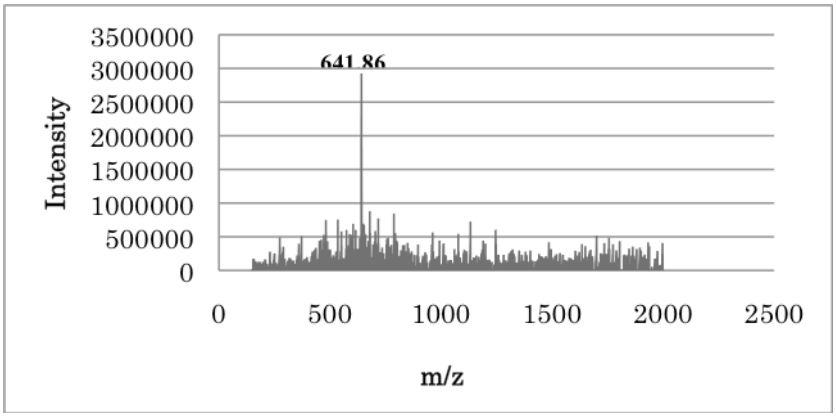

Fig. S2. ESI-MS spectrum of DOTA-Gd-G_2R_8 after HPLC purification; MS (m/z) calculated for $C_{68}H_{127}GdN_{38}O_{18}$ 1921.94, found $[M + 3H^+]$ = 641.86

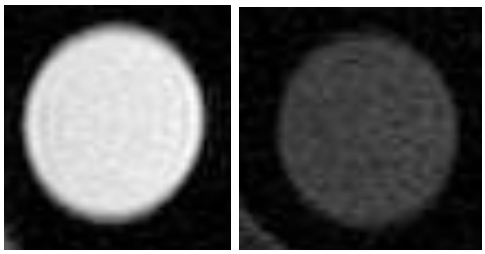

Fig. S3. Spin echo images (TR = 500 ms, TE = 14 ms) of an aqueous solution of DOTA-Gd-G_2R_8 (2.5 mM, left) and phosphate buffer saline without contrast agent (right)

a) b)

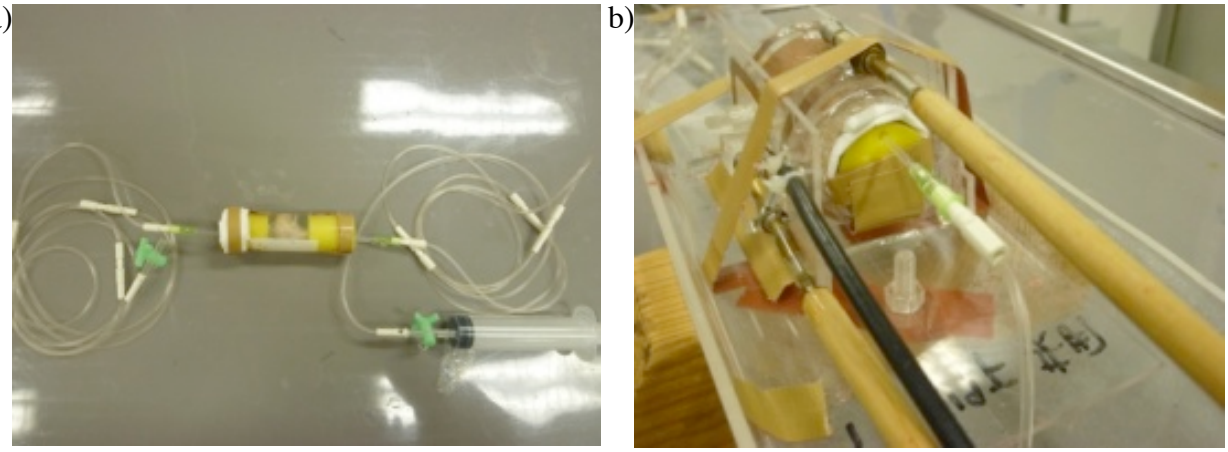

Fig. S4. Apparatus used for MR *ex vivo* imaging of a rabbit femoral condyle.

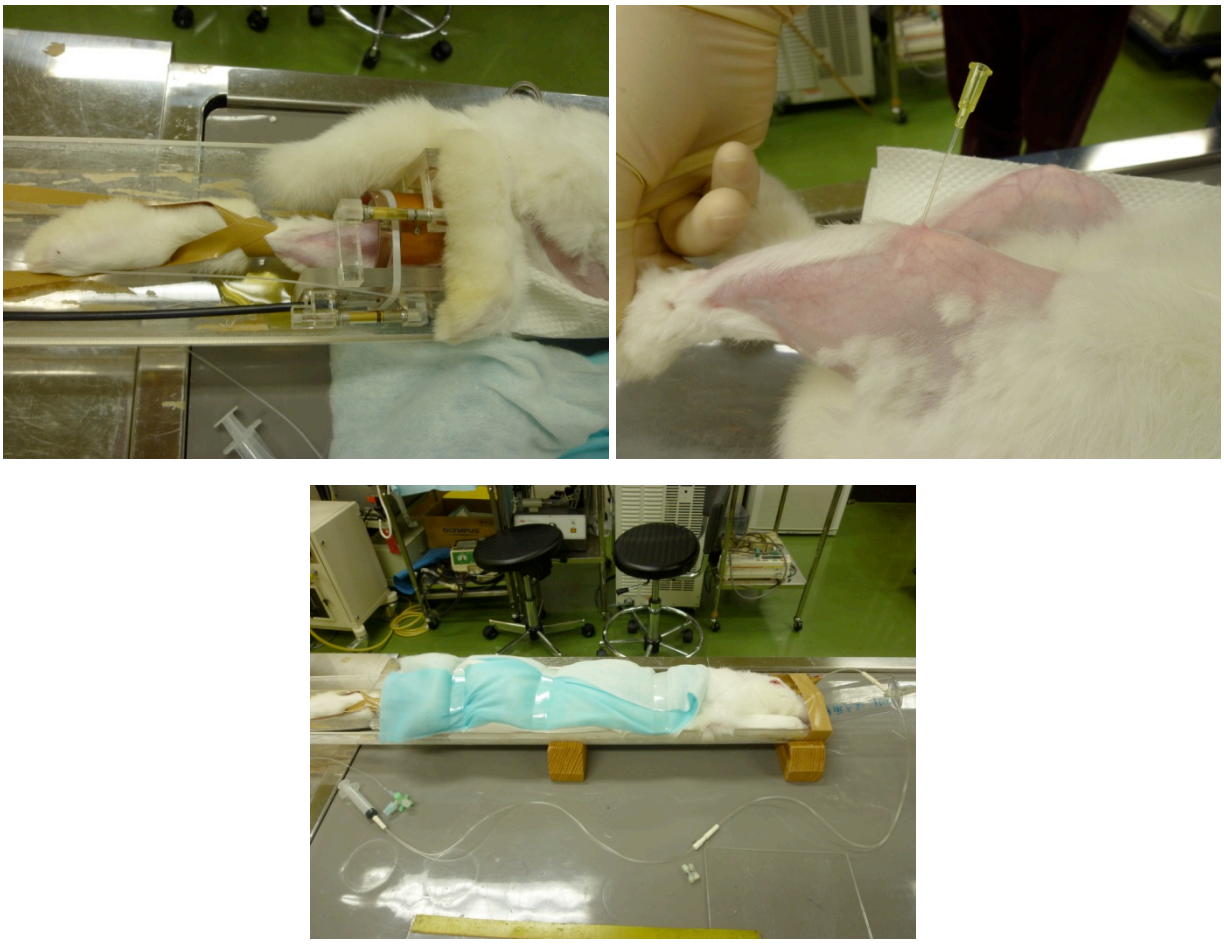

Fig. S5. *In vivo* imaging of a rabbit articular cartilage with DOTA-Gd-G_2R_8.