LDL-based nanoparticles for contrast enhanced MRI of atheroplaques in mouse models

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Supporting Information

Contents

1. Syntheses of GdDO3A-OA (Gd2) and fluorescein-OA (3)

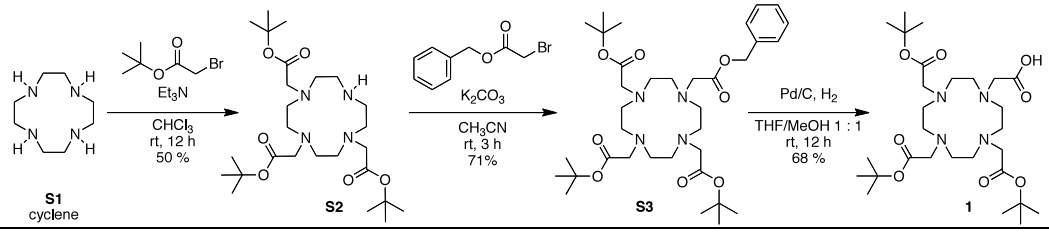
General: Melting points were measured with MELTEMP (Laboratory Devices, Holliston, MA, USA) and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance AVII-500 NMR spectrometer (Bruker, Billerica, MA, USA) respectively at 500 and 125 MHz. Low resolution ESI-MS were performed on a Waters SQD equipped with an Acquity UPLC (Waters, Milford, MA, USA). High resolution ESI-MS were performed on a Waters LCT Premier XE LC/MS system and a Waters GC-TOF Premier (Waters). MALDI-TOF-MS were performed on a Bruker MALDI-TOF-TOF (Bruker). The IR spectra were recorded on the JASCO FT/IR-4100 (JASCO, Tokyo, JPN).

All the reagents were purchased from the corresponding commercially available sources and were purified as described when needed. All solvents were purchased from EMD Chemicals Inc. (Gibbston, NJ, USA) unless described. THF, acetonitrile, and methanol were passed over activated alumina under an Ar atmosphere and DMF was passed over molecular sieves under an Ar atmosphere by a solvent system (Innovative Technology Inc., Newburyport, MA, USA). Benzene and dichloromethane were distilled over calcium hydride. Column chromatography, analytical TLC, and preparative TLC were performed respectively on EMD Silica Gel 60 (230–400 mesh), EMD precoated plates (silica gel 60 F_{254}, Art 5715, 0.25 mm), and plates prepared from EMD silica gel (silica gel 60 FP_{254}, Art 7749). TLC plates were visualized by UV light, a phosphomolybdic acid stain, or a $KMnO_4$ stain. HPLC analyses were carried out with a PU-2080 plus pump (JASCO), a UV-2077 plus UV-Vis detector (JASCO), and an NQAD detector (GRACE, Deerfield, IL, USA) using a data analysis system EZChrom (JACSO).

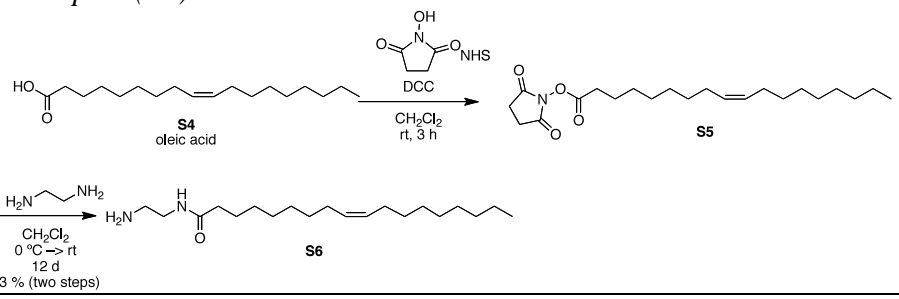
1-1. Synthesis of GdDO3A-OA (Gd2) for the modification of LDL nanoparticle

Scheme S1. Synthesis of GdDO3A-OA (Gd**2**)

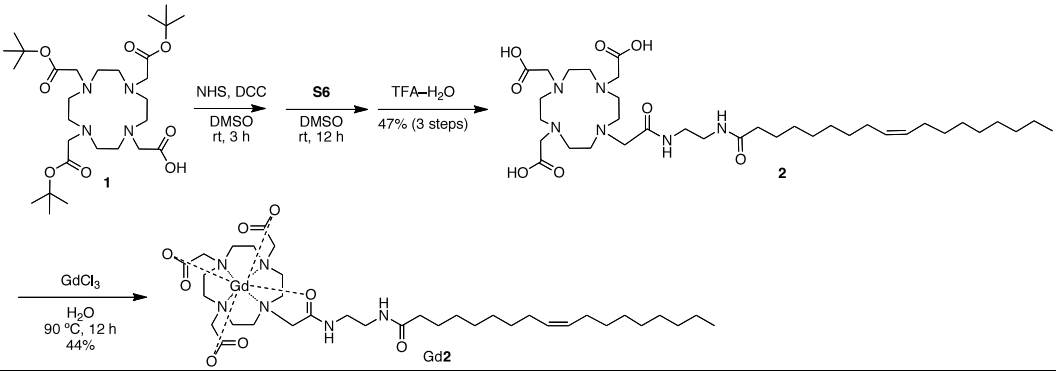
*Sythesis of DOTA part (**1**)*



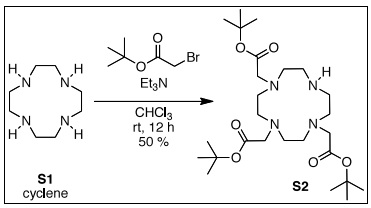
*Synthesis of oleic acid part (**S6**).*



Conjugation of DO3A part and oleic acid part and subsequent chelating of Gd(III).



1-1-1. Synthesis of the DOTA derivative 1.



Tri-*tert*-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (S2).[1] To a solution of 1,4,7,10-tetraazacyclododecane **S1** (cyclene) (400 mg, 2.32 mmol) and triethylamine (2.3 g, 23.2 mmol, 10 eq.) in anhydrous chloroform (40 mL), *tert*-butyl bromoacetate (1.48 g, 7.6 mmol, 3.3 eq.) in anhydrous chloroform (10.0 mL) was added dropwise under argon atmosphere. The reaction mixture was stirred for 16 hrs at room temperature. The resulting solution was washed by water (3×40 mL) and the organic phase was dried over Na_2SO_4. TLC analysis of crude extract gave two spots at R_f 0.50 and 0.40 ($CHCl_3$–EtOH (9:1)). After the solvent was evaporated, the crude products were purified by Al_2O_3 column chromatography ($CHCl_3$–EtOH (9:1)) to give a colorless solid **S2** (471 mg (0.92 mmol, y = 39%). ^{1}HNMR (500 MHz, $CDCl_3$) δ 3.97 (s, 4H, N–C**H$_2$**–COO), 3.29 (s, 2H, N–C**H$_2$**–COO), 3.11 (br.s, 4H, N–C**H$_2$**–CH_2–N), 2.93 (m, 8H, N–C**H$_2$**–C**H$_2$**–N), 2.88 (br.s, 4H,

[1] (a) A. Dadabhoy, S. Faulkner and P. G. Sammes, *J. Chem. Soc., Perkin Trans.,* 2002, **2**, 348. (b) C. Wängler, B, Wängler, M. Eisenhut, U. Haberkorn and W. Mier, *Bioorg, Med. Chem.,* 2008, **16**, 2606. (c) N. Kamaly, T. Kaler, A. Ahmad, M. H. Oliver, P.-W. So, A. H. Herlihy, J. D. Bell, M. R. Jorgensen and A. D. Miller, *Bioconjugate Chem.*, 2008, **19**, 118.

N–C**H$_2$**–CH$_2$–N), 1.48 (s, 18H, ^{t}Bu), 1.46 (s, 9H, ^{t}Bu); MS (ESI+) *m/z* calcd for $C_{26}H_{50}N_4O_6$: 514.4; found: 515.3 ([M+H]$^+$).

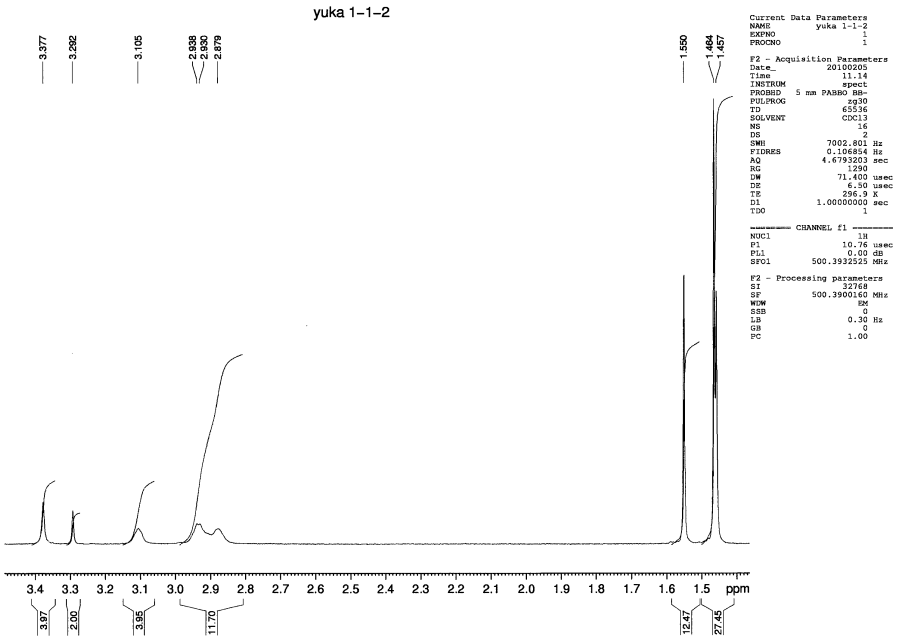


Figure S1. ^{1}H-NMR spectrum of compound **S2**.

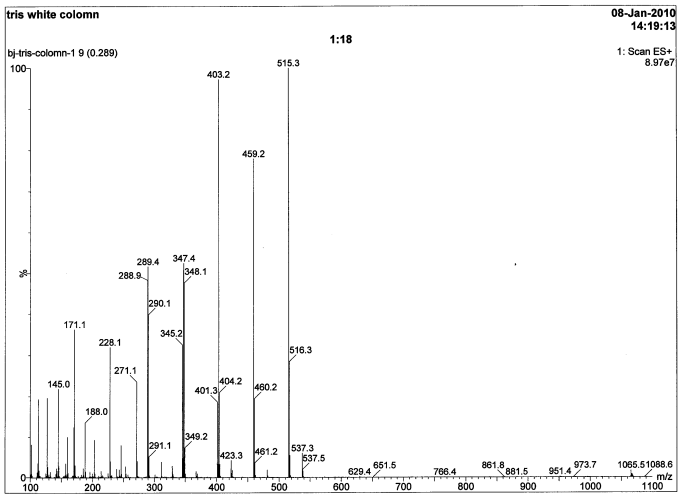
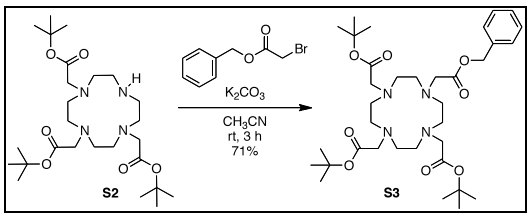


Figure S2. ESI-MS spectrum of compound **S2**.



Tri-*tert*-butyl 2,2',2''-(10-(2-(benzyloxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (S3).[1] To a suspension of **S2** (700 mg, 1.36 mmol) in acetonitrile, K_2CO_3 powder (280 mg, 2.03 mmol, 1.5 eq.) and subsequently benzyl bromoacetate (373 mg, 1.63 mmol, 1.2 eq.) were added. Reaction was stirred at room temperature for 3 hrs. Reaction process was monitored by TLC ($CHCl_3$–EtOH (9:1), **S3**: R_f = 0.8). The precipitated solids were removed by filtration and the filtrate was concentrated to give the crude product, which was purified by silica gel column chromatography using CH_2Cl_2–MeOH (100:0 -> 90:10) to give colorless solids **S3** (638 mg, 0.962

mmol, y = 71%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 5H, Ph), 5.11 (s, 2H, –C**H$_2$**–Ph), 2.0–3.7 (br.m, 24H, N–**CH$_2$**–COO, N–**CH$_2$–CH$_2$**–N), 1.44 (s, 9H, ^{t}Bu), 1.43 (s, 18H, ^{t}Bu); MS (ESI+) *m/z* calcd. for $C_{35}H_{58}N_4O_8$: 662.4; found: 663.4 ([M+H]$^+$), 685.3 ([M+Na]$^+$).

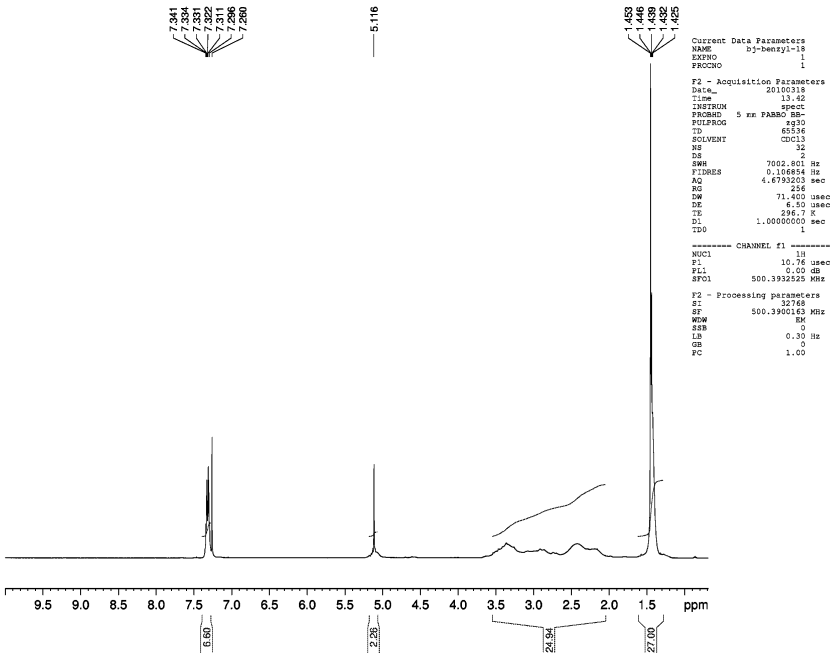
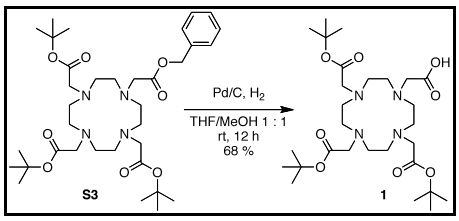


Figure S3. ^{1}H-NMR spectrum of compound **S3**.



2-(4,7,10-Tris(2-(*tert*-butoxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecan-1-yl)acetic acid (1, commercially available now).[1] To a solution of **S3** (83 mg, 0.13 mmol) in 5 mL THF-MeOH (1:1), palladium on carbon (10% Pd, 11.4 mg) was added and the reaction mixture was stirred under hydrogen atmosphere at room temperature for 12 hrs. The reaction was monitored by TLC (CH_2Cl_2–MeOH (1:1) (0.1% water), **1**: R_f = 0.5). After completion of the reaction, the catalyst was removed by filtration, the solvents were evaporated, and the crude mixture was rapidly purified by silica gel column chromatography (CH_2Cl_2 -> CH_2Cl_2–MeOH (0.1% water)). The product was obtained as a colorless solid **1** (50.7 mg, 0.089 mmol, 68%). ^{1}H NMR (500MHz, CD_2Cl_2) δ 2.0–3.7 (br.m, 24H, N–**CH$_2$**–COO, N–**CH$_2$–CH$_2$**–N), 1.47 (s, 27H, ^{t}Bu); MS (ESI–) *m/z* calcd. for $C_{28}H_{52}N_4O_8$: 572.38; found: 571.57 ([M–H]$^-$).

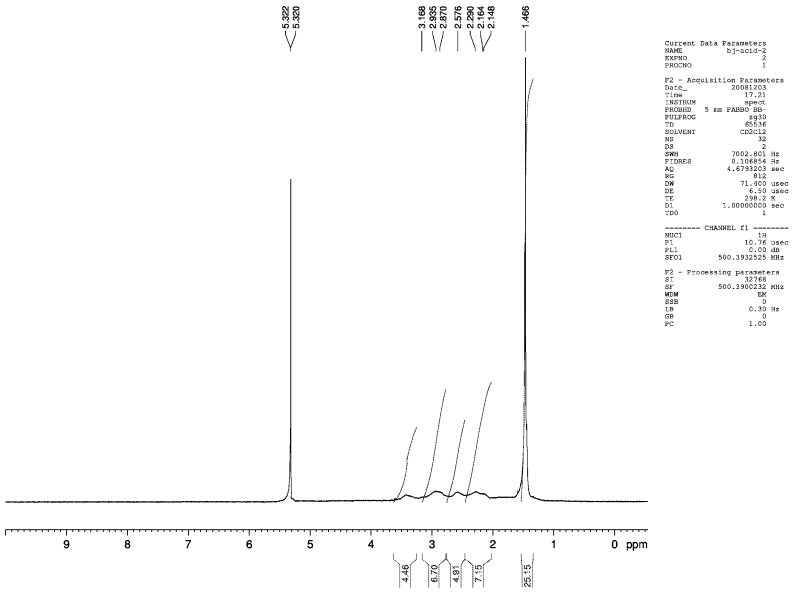
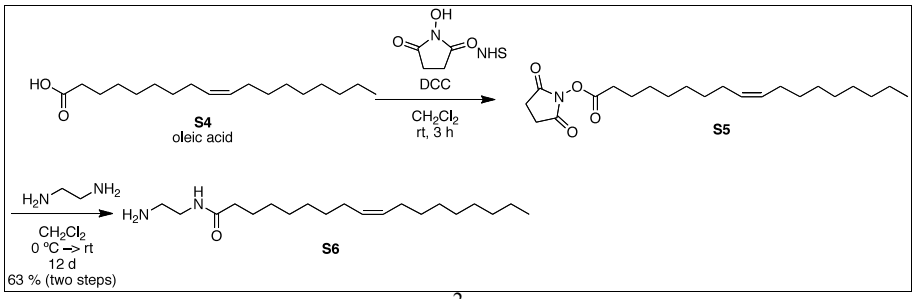


Figure S4. ^{1}H-NMR spectrum of compound **1**.

1-1-2. Synthesis of oleic acid linker part 2.



***N*-(2-Aminoethyl)oleamide (S6).**[2] The oleic acid (**S4**, 2.0 g, 7.08 mmol) and 0.8 g of *N*-hydroxysuccinimide (NHS, 0.8 g, 6.95 mmol) were mixed in dry CH_2C1_2 (40 mL) at room temperature. Subsequently, dicyclohexylcarbodiimide (DCC, 1.45 g, 7.03 mmol), in dry CH_2C1_2 (5 mL) was added slowly to the suspension while rapid stirring. The mixture was stirred for 3 hrs at room temperature. The precipitated dicyclohexylurea was removed by filtration and the filtrate was diluted with dry CH_2C1_2 (100 mL). The obtained solution was added dropwise to a vigorously-stirred solution of ethylenediamine (12.75 g, 212 mmol) in CH_2C1_2 (33 mL) at 0 °C. The reaction mixture was allowed to reach to room temperature and stirred for another 12 hrs. The reaction mixture was filtered, concentrated under vacuum to approximately 50 mL, and washed with a 10% sodium chloride solution (24 ml) four times. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo* to give colorless solids **S6** (1.45 g, 4.47 mmol, y = 63%, 2 steps). IR (neat) [cm^{-1}] 3300m, 2917s, 2844s, 1639s, 1560m, 1467w, 1261w, 904w, 717w cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 5.98 (br.s, 1H, CH_2–N**H**–CO), 5.33 (m, 2H, –C**H**=C**H**–), 3.30 (q, J = 11.5, 5.8 Hz, 2H, CH_2–C**H$_2$**–NH–CO), 2.83 (t, J = 5.9 Hz, 2H, H_2N–C**H$_2$**–CH_2), 2.18 (t, J = 7.9, 7.4 Hz, 2H, –CO–C**H$_2$**–), 1.9-2.1 (m, 4H, –C**H$_2$**–CH=CH–C**H$_2$**–), 1.62 (br.t, J = 6.8 Hz, 2H, –C**H$_2$**–), 1.2–1.5 (m, 20H, –C**H$_2$**–), 0.82 (t, J = 7.0, 6.8 Hz, 3H, –C**H$_3$**); HRMS (ESI+) *m/z* calcd. for $C_{20}H_{40}N_2ONa$: 347.3038, found: 347.3051 $[M + Na]^+$.

[2] V. Derue, S. Alexandre, H. Huguet, B. Deschrevel and J. M. Vallenton, *Thin Solid Films*, 1997, **306**, 1.

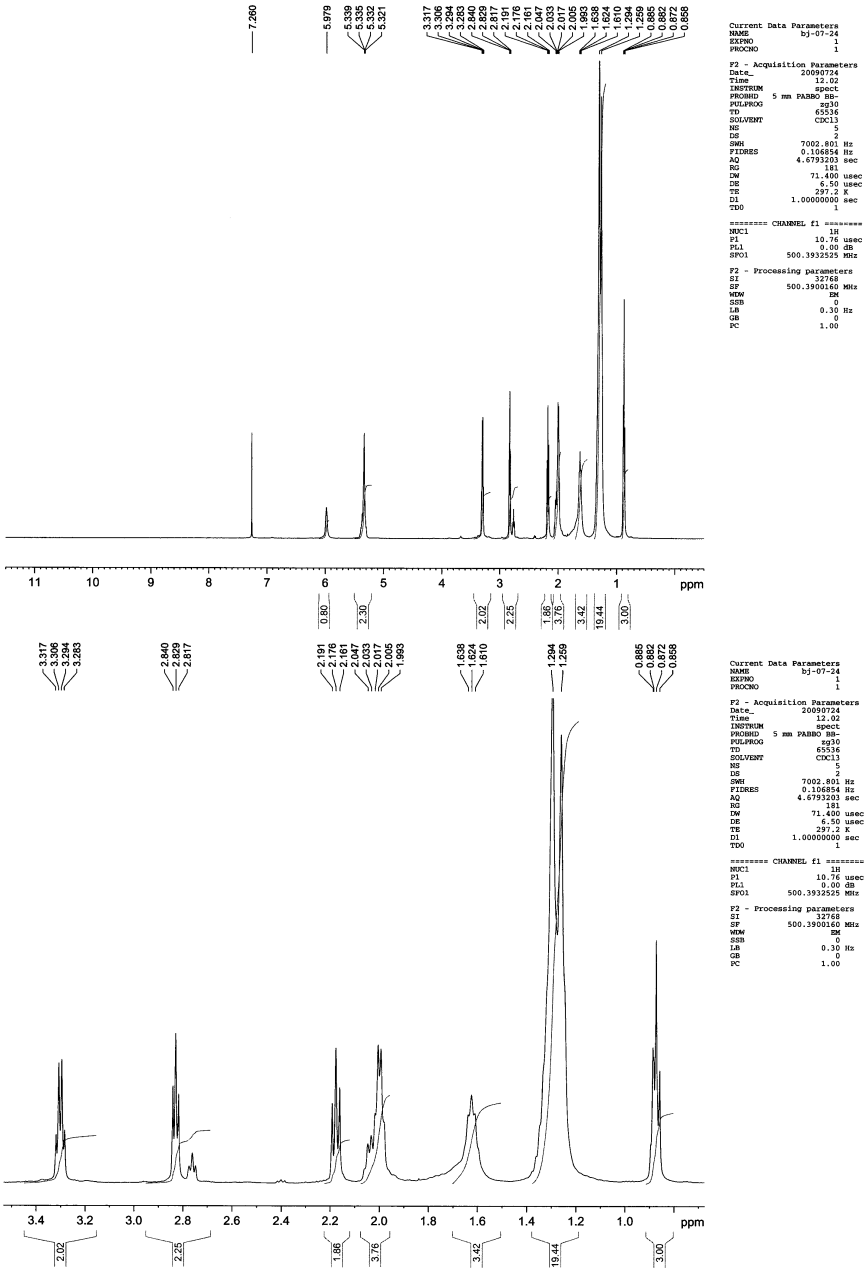


Figure S5. ^{1}H-NMR spectrum of compound **S6**.

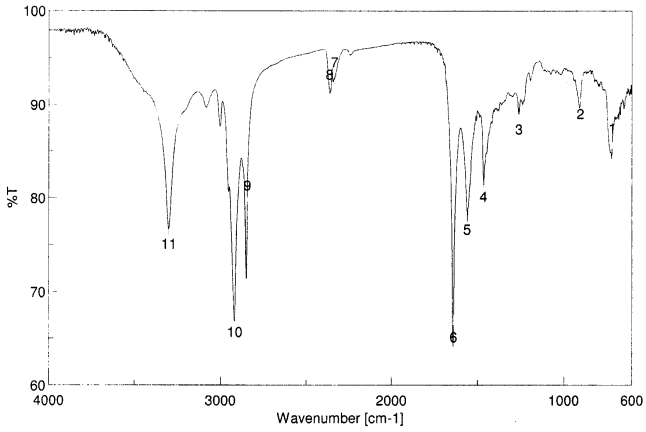
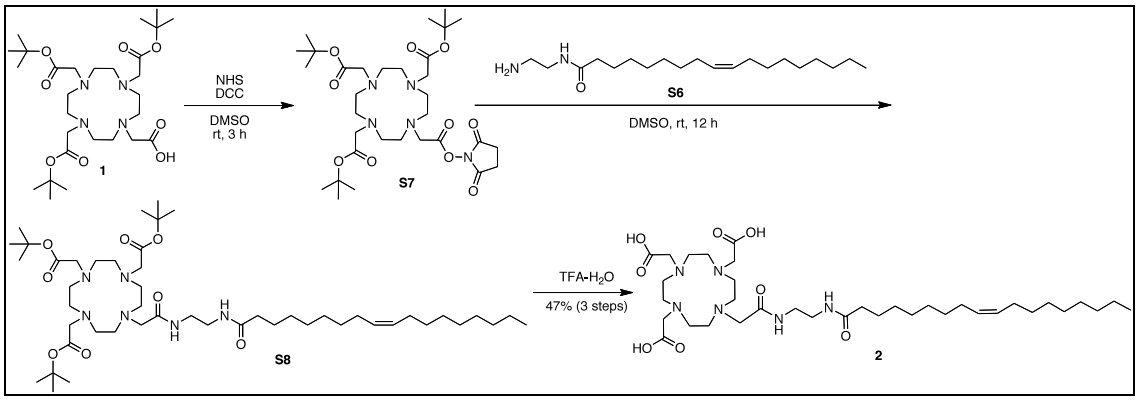


Figure S6. FT-IR spectrum of compound **S6**.

1-1-3. Conjugation of DOTA part 1 with oleic acid linker S6 and subsequent Gd-chelation to give probe GdDO3A-OA (Gd2).



(*Z*)-2,2',2''-(10-(2-((2-oleamidoethyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (2). To a mixture of cyclene derivative **1 (**300 mg, 0.52 mmol) and NHS (115 mg, 1.0 mmol) in dry DMSO (30 mL), DCC (200 mg, 0.97 mmol) solution in dry DMSO was added slowly while stirring rapidly at room temperature. The reaction mixture was stirred subsequently for 3 hrs. The precipitated dicyclohexylurea was removed by filtration and the filtrate was diluted with dry DMSO (10 mL). The diluted solution was added dropwise to a vigorously stirred solution of oleic acid derivative **S6** (170 mg, 0.52 mmol) in DMSO (10 mL) and stirred for 12 hrs. The reaction progress was monitored by TLC. Upon completion of the reaction, DMSO was removed by lyophilizing of the reaction mixture. The reaction mixture was passed through a short silica gel column, treated with TFA–H_2O and purified by HPLC (column: YMC-Pack ODS-A, di 20 mm x 250 mm, YMC Co., Ltd., Kyoto, Japan, solvent: gradient H_2O–CH_3CN (5-95%), flow rate: 10 mL/min). Compound **2** was eluted in the retention time around 20 min. The eluate was collected 10 mL per fraction, which were analyzed by MALDI-TOF-MS. The fractions contains **2** were collected and concentrated *in vacuo* to subject to subsequent lyophilizing to give colorless solids **2** (174 mg, 0.24 mmol, y = 47%, 3 steps). IR (neat) [cm^{-1}] 3417m, 2994s, 2922s, 2863m, 2713m, 1678s, 1469w, 1430w, 1397w, 1207s, 1144s, 795w, 721w; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.09 (br.s, 1H, –N**H**–), 6.73 (br.s, 1H, –N**H**–), 5.32 (m, 2H, –C**H**=C**H–**), 3.50 (m, 2H, CO–NH–C**H_2**–CH_2), 3.41 (m, 2H, CH_2–C**H_2**–NH–CO), 2.99 (br.s, 24H, N–**CH_2**–COO, N–**CH_2–CH_2**–N), 2.23 (t, J = 7.3 Hz, 2H, CH_2C**H_2**CONH), 1.99 (q, 4H, –C**H_2**CH=CHC**H_2**–), 1.57-1.60 (m, 4H, –C**H_2**–), 1.26-1.28 (m, 18H, –C**H_2**–), 0.86 (t, J = 6.5 Hz, 3H, –C**H_3**); HRMS (ESI+) *m/z* calcd for $C_{36}H_{66}N_6O_8$: 710.4942, found: 711.5007 $[M+H]^+$.

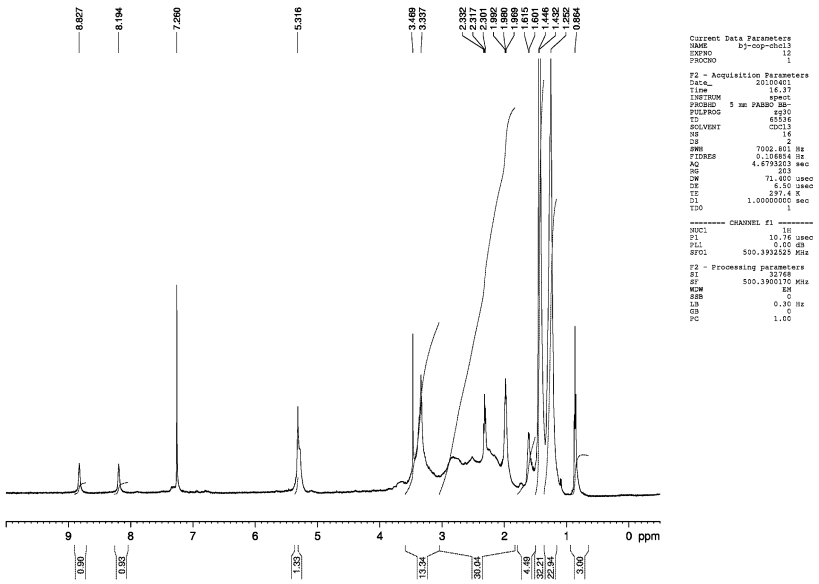


Figure S7. ^{1}H-NMR spectrum of compound **S8**.

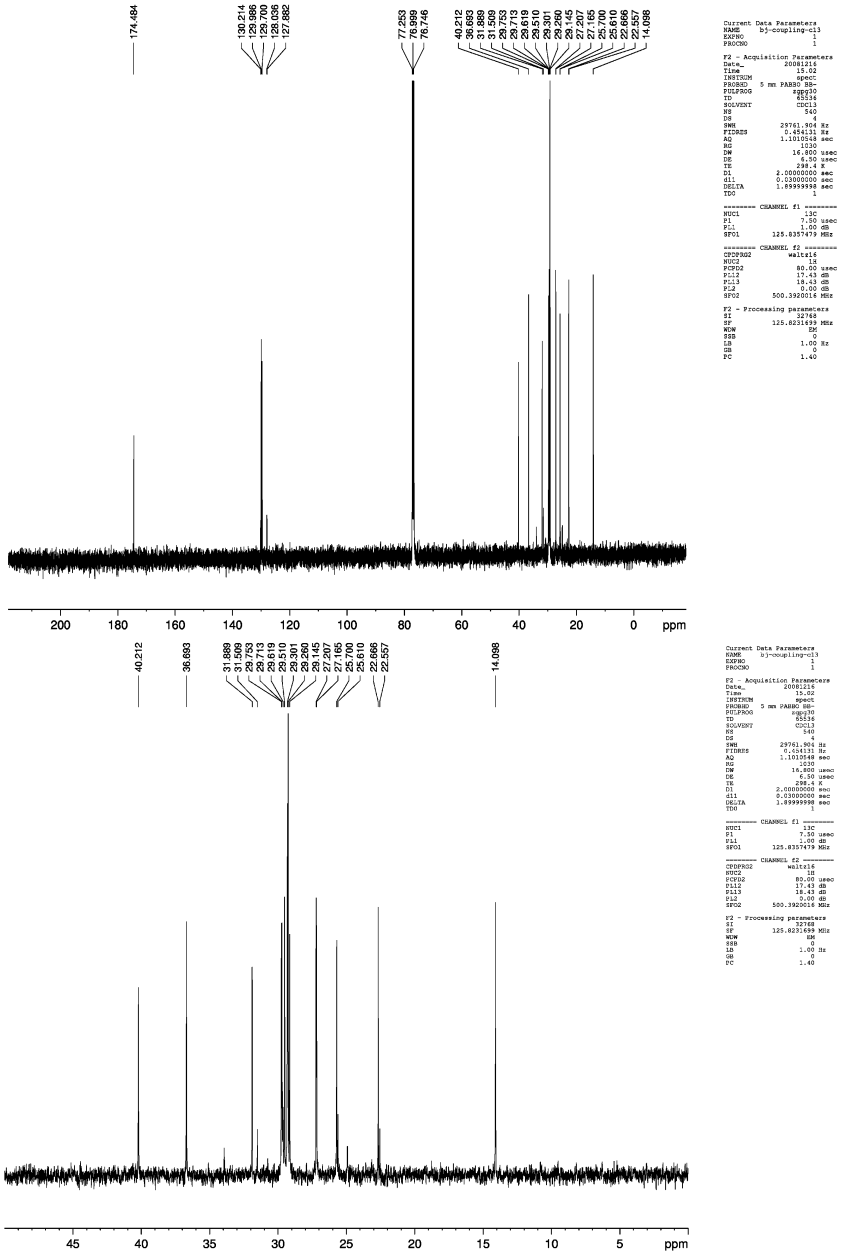


Figure S8. ^{13}C-NMR spectrum of compound **S8**.

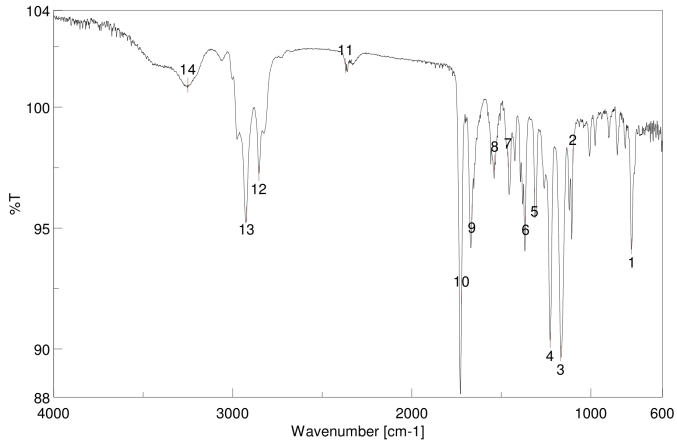


Figure S9. FT-TR spectrum of compound **S8.**

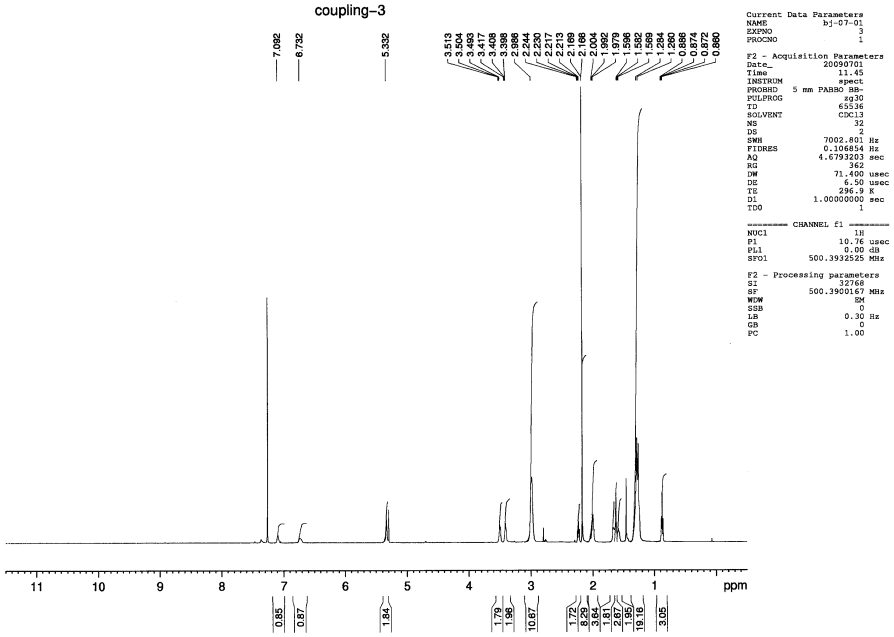


Figure S10. ^{1}H-NMR spectrum of compound **2**.

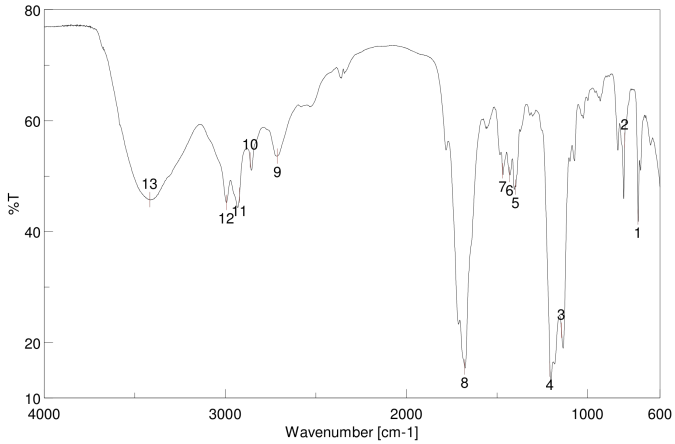


Figure S11. FT-TR spectrum of compound **2**.

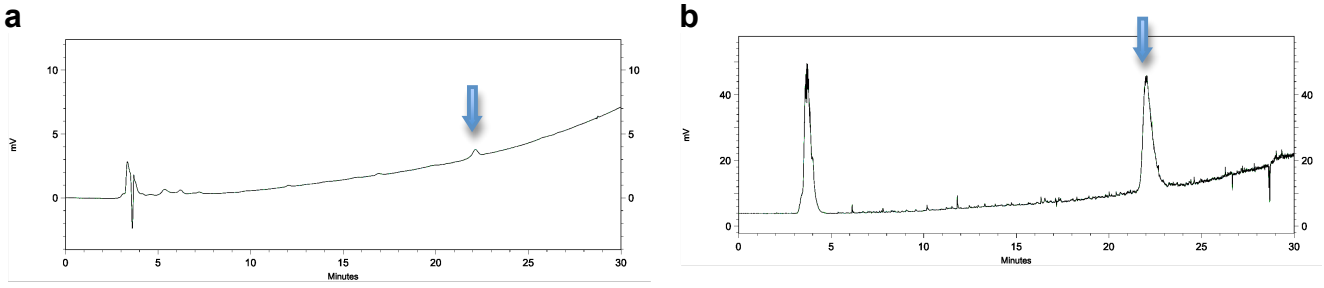
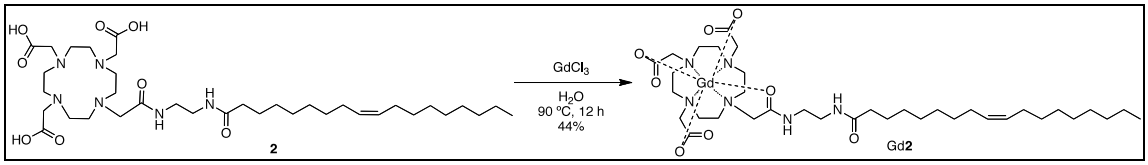


Figure S12. HPLC analysis of **2** by analytical condition. Column: YMC-Pack ODS-A (id 4.6 mm x 250mm), solvent: H_2O-MeCN (5-95%, over 20min), flow rate: 1.0 mL/min, detection: UV 254 nm (a) and NQAD (b).



A mixture of $GdCl_3$•$6H_2O$ (79.5 mg, 0.214 mmol) and **2** (152 mg, 0.214 mmol) was stirred in distilled H_2O (20 mL) at 90 °C overnight. The water was removed by freeze-drying to give a crude

colorless powder (220 mg). A portion of this material (100 mg) was dissolved in 50% MeOH/water and adsorbed onto C-18 derivatized silica gel. Elution (0-100% MeOH/water) and freeze-drying resulted in a colorless power (67.6 mg) of which 50 mg was subsequently dissolved in methanol (2 mL) and purified by HPLC ((column: YMC-Pack ODS-A, diameter 20 mm x 250 mm, YMC Co., Ltd., solvent: gradient H_2O–CH_3CN (5-95%), flow rate: 10.0 mL/min, Gd**2** was eluted at 28-29 min)) to give a colorless wax Gd**2** (27.3 mg, 0.0316 mmol, y = 44%). IR (neat) [cm^{-1}] 3291m, 3077m, 2936s, 2849s, 1727w, 1649m, 1557m, 1450w, 1402w, 1207m, 994w, 804w, 746w, 726w; HRMS (ESI+) *m/z* calcd. for $C_{36}H_{63}N_6O_8{}^{155}Gd$: 862.3933, found: 862.3934 (M^+), calcd. for $C_{36}H_{63}N_6O_8{}^{156}Gd$: 863.3928, found: 863.3937 (M^+), calcd. for $C_{36}H_{63}N_6O_8{}^{157}Gd$: 864.3947, found: 864.3954 (M^+), calcd. for $C_{36}H_{63}N_6O_8{}^{158}Gd$: 865.3948, found: 865.3958 (M^+), calcd. for $C_{36}H_{63}N_6O_8{}^{160}Gd$: 867.3978, found: 867.3982 (M^+), calcd. for $C_{36}H_{63}N_6O_8{}^{155}GdNa$: 885.3831, found: 885.3832 ($[M+Na]^+$), calcd. for $C_{36}H_{63}N_6O_8{}^{156}GdNa$: 886.3826, found: 886.3835 ($[M+Na]^+$), calcd. for $C_{36}H_{63}N_6O_8{}^{157}GdNa$: 887.3845, found: 887.3852 ($[M+Na]^+$), calcd. for $C_{36}H_{63}N_6O_8{}^{158}GdNa$: 888.3846, found: 888.3855 ($[M+Na]^+$), calcd. for $C_{36}H_{63}N_6O_8{}^{160}GdNa$: 890.3876, found: 890.3880 ($[M+Na]^+$), (ESI–) *m/z* calcd. for $C_{36}H_{63}N_6O_8{}^{155}GdCl$: 897.3622, found: 897.3623 ($[M+Cl]^-$), calcd. for $C_{36}H_{63}N_6O_8{}^{156}GdCl$: 898.3617, found: 898.3625 ($[M+Cl]^-$), calcd. for $C_{36}H_{63}N_6O_8{}^{157}GdCl$: 899.3636, found: 899.3635 ($[M+Cl]^-$), calcd. for $C_{36}H_{63}N_6O_8{}^{158}GdCl$: 900.3637, found: 900.3636 ($[M+Cl]^-$), calcd. for $C_{36}H_{63}N_6O_8{}^{160}GdCl$: 902.3667, found: 902.3654 ($[M+Cl]^-$).

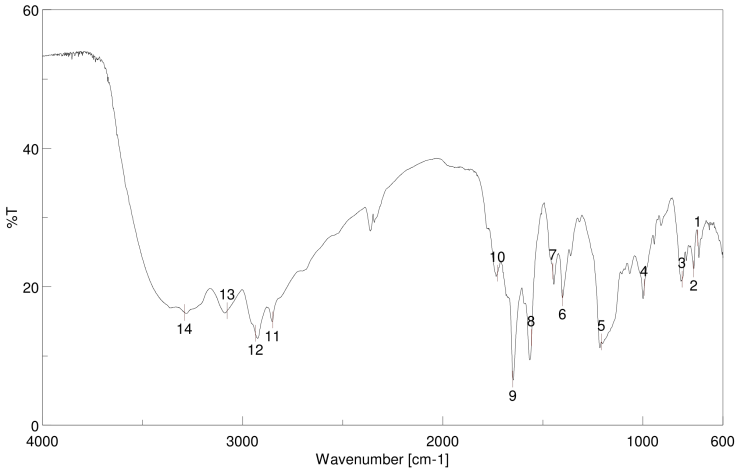


Figure S13. FT-IR spectrum of compound Gd**2**.

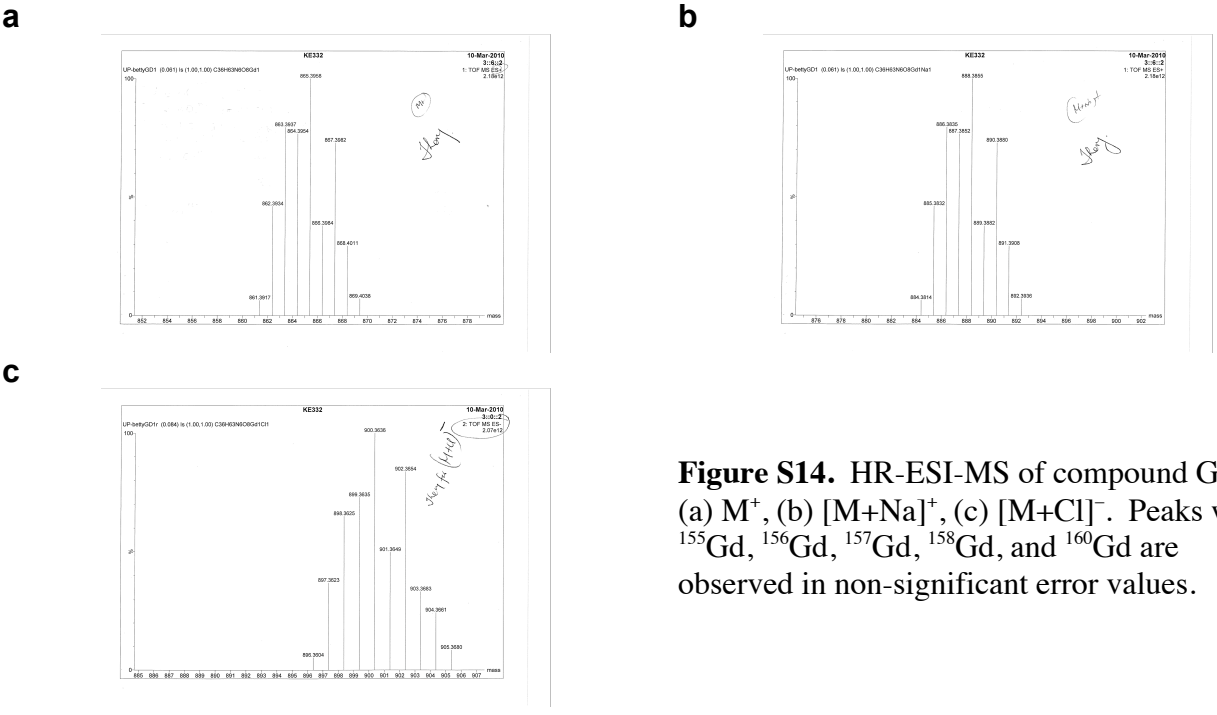


Figure S14. HR-ESI-MS of compound Gd**2**. (a) M^+, (b) $[M+Na]^+$, (c) $[M+Cl]^-$. Peaks with ^{155}Gd, ^{156}Gd, ^{157}Gd, ^{158}Gd, and ^{160}Gd are observed in non-significant error values.

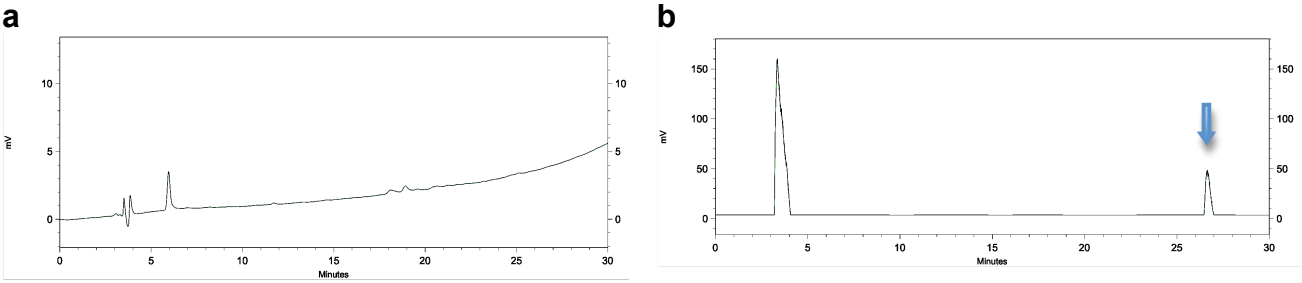
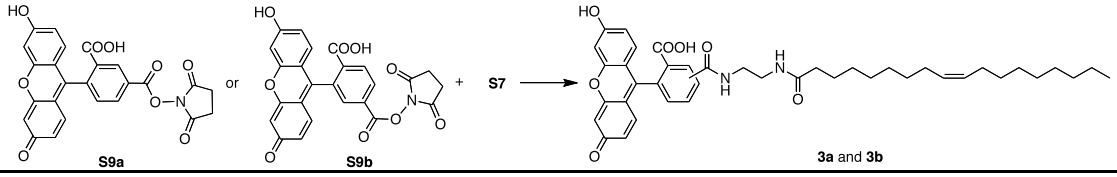


Figure S15. HPLC analysis of Gd**2** by analytical condition Column: YMC-Pack ODS-A (id 4.6 mm x 250mm), solvent: H_2O-MeCN (5-95%, over 20min), flow rate: 1.0 mL/min, detection: UV 254 nm (a) and NQAD (b).

1-2. Synthesis of fluorescein-OA (3) for the modification of LDL nanoparticle

Scheme S4. Synthesis of Fluorescin-OA **3**.



A mixture of NHS-fluorescein **S9a** and **b** (34.7 mg, 0.073 mmol, Thermo Scientific, Rockford, IL, USA) and amine **S7** (25.8 mg, 0.079 mmol) in DMF (5 mL) was stirred overnight at room temperature. The DMF was removed *in vacuo* and crude mixture was purified by silica gel column chromatography (CH_2Cl_2–MeOH–H_2O (90:10:1)) and by subsequent HPLC (column: YMC-Pack ODS-A, di 20 mm x 250 mm, detectors (UV): 220, 254, 301 nm, solvent: gradient MeCN–H_2O (5-95%, over 30 min), flow rate: 10.0 mL/min, rt of **3a** and **3b**: 15.6 min) to give yellow solids 27.8 mg (**3a, 3b** mixture, 52%). IR (neat) [cm^{-1}] 3441m, 3228m, 2961m, 1645m, 1547w, 1460w, 1062m, 1027m, 877w, 780w; ^{1}H NMR (500 MHz, MeOH-d_4): δ 8.46 (s, 0.6H, Ar**H** *ortho* to COOH in **5a**), 8.20 (d, J = 8.5 Hz, 0.6H, Ar**H** *para* to COOH in **5a**), 8.12 (m, 0.8H, Ar**H** *ortho* to COOH and Ar**H** *meta to* COOH in **3b**), 7.63 (s, 0.4H, Ar**H** *meta* to COOH in **3b**), 7.31 (d, J = 8.0 Hz, 0.6H, Ar**H** *meta* to COOH in **3a**), 6.74 (brs, 2H, Ar**H** in xanthene in **3a** and **3b**), 6.59-6.65 (m, 4H, Ar**H** in xanthene in **3a** and **3b**), 5.31 (m, 2H, –C**H**=C**H**– in **3a** and **3b**), 3.55 (brm, 1.6H, CO–NH–C**H**$_2$–C**H**$_2$–NH–CO in **3b**), 3.46 (brm, 2.4H, CO–NH–C**H**$_2$–C**H**$_2$–NH–CO in **3a**), 2.20 (m, 1.2H, –NH–CO–C**H**$_2$–CH_2 in **3a**), 2.09 (m, 0.8H, NH–CO–C**H**$_2$–CH_2 in **3b**), 1.99 (brs, 4H, –C**H**$_2$CH=CHC**H**$_2$– in **3a** and **3b**), 1.28-1.59 (brm, 22H, –C**H**$_2$– in **3a** and **3b**), 0.89 (brm, 3H, C**H**$_3$ in **3a** and **3b**), ^{13}C NMR (125 MHz, MeOH-d_4) δ 177.0, 170.1, 168.5, 162.9, 154.9, 153.6, 142.0, 137.9, 135.1, 130.9, 130.77, 130.75, 130.6, 130.4, 126.4, 118.3, 114.7, 111.7, 103.6, 41.2, 39.9, 37.2, 33.1, 30.82, 30.76, 30.6, 30.4, 30.34 (2C), 30.30, 30.27, 30.2, 28.1, 27.0, 23.7, 14.5, HRMS (ESI–) m/z calcd for $C_{41}H_{50}N_2O_7$ 682.3618, found 681.3553 [M–H]$^-$.

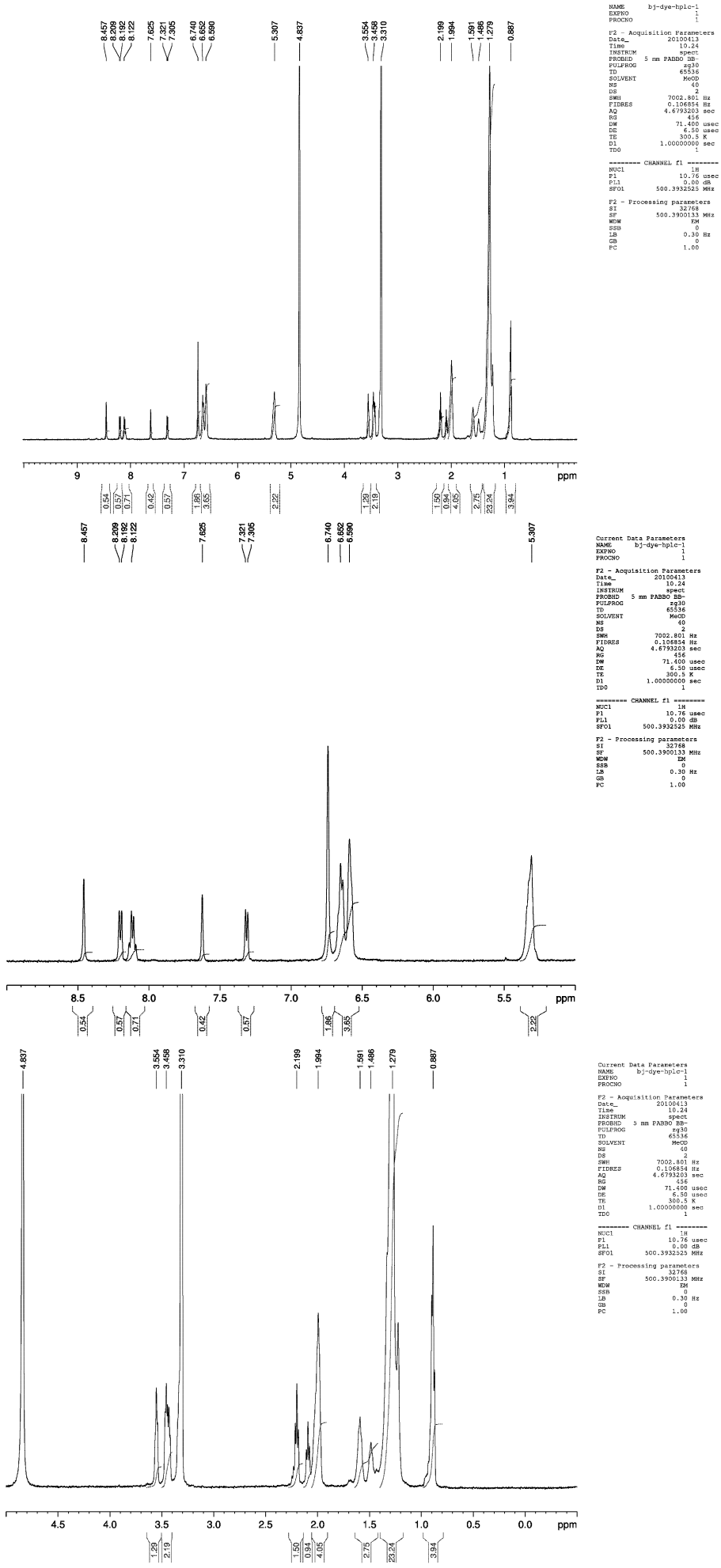


Figure S16. ^{1}H-NMR spectrum of compounds **3a** and **3b**.

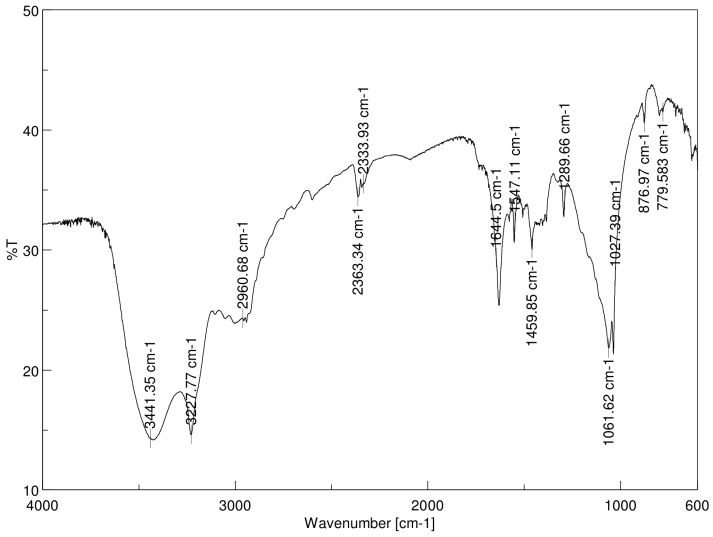


Figure S17. FT-IR spectrum of compounds **3a** and **3b**.

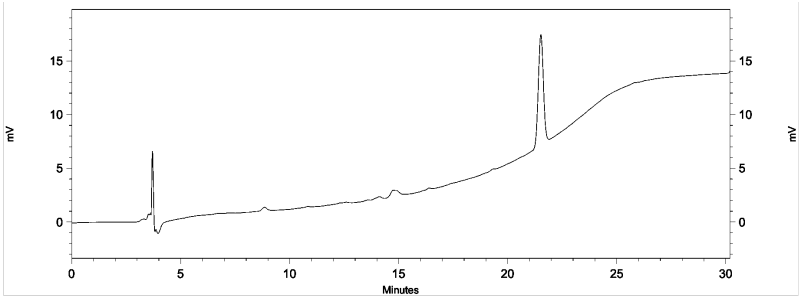


Figure S18. HPLC analysis of purified **3a** and **3b** by analytical condition Column: YMC-Pack ODS-A (id 4.6 mm x 250mm), solvent: H_2O-MeCN (5-95%, over 20min), flow rate: 1.0 mL/min, detection: 254 nm.

2. Modification of LDL particles with fluorescein-OA (3) or GdDO3A-OA (Gd2)

2-1. Modification of LDL with fluorescein-OA (3).

LDL source and dialysis: The LDL, which was isolated from fresh plasma of healthy donors by sequential ultracentrifugation, was purchased from Dr. Lund-Katz at the Children Hospital of Philadelphia. The obtained suspension of LDL was dialyzed against PBS(–) overnight at 4 ºC using dialysis bags (SpectralPor molecularporous membrane tubing, flat width of 25 nm and a MWCO 12-14000), which was previously activated by microwave for 2 min in milliQ water. The PBS(–) outside of the dialysis bag was changed after first 2 hours of dialysis.

Quantification of LDL by Lowry assay: The concentration of dialyzed LDL was estimated by total protein analysis using a commercial Lowry protein assay kit (Sigma-Aldrich, St. Louis, MO) based on the assumption that ApoB-100 (550 kDa) was the only protein present per LDL particle. With a standard curve of Lowry test using BSA, the concentration of LDL was determined as 1.9-2.4 mg/mL depending on the each sample lot provided by Dr. Lund-Katz.

Intercalation of fluorescein-OA (3) into LDL particle: To a dialyzed LDL suspension in PBS(–), fluorescein-OA (**3**) solution in PBS(–) were added in each molecular ratio (1:0, 50, 100, 200, and 500) and mixture were shaken for 2 hours at 37 ºC under N_2 atmosphere. After the modification, LDL was purified by dialysis to remove free fluorescein-OA in the solution.

2-2. Modification of LDL with GdDO3A-OA (Gd2)

To a dialyzed LDL suspension in PBS(–), GdDO3A-OA (Gd**2**) solution in DMSO-PBS(–) (3:7) were added in each molecular ratio (1:0, 50, 100, 200, and 500) and mixture were shaken for 2 hours at 37 ºC under N_2 atmosphere. After the modification, LDL was filtered to remove the precipitation (mainly Gd**2**) and subsequently purified by dialysis (200 mL PBS, 4 °C, 2 h then fresh 200 mL PBS, 4 °C, overnight) to remove unbound Gd**2** in the solution. Particle integrity was analyzed by Light Scattering as described below.

2-3. General characterization of LDL nanoparcicles

Light scattering: Particle size of modified/unmodified LDL was analyzed by a light scattering photon correlation spectroscopy Zetasizer 3000HSA (Malvern Instruments Ltd., Worcestershire, UK) equipped with a 10-mW He-Ne laser operating at 633 nm and a detector angle of 90º. The data were modeled assuming spherical particles undergoing Brownian motion.

UV-Vis absorption: UV-Vis spectra were recorded on UV-Vis/NIR Spectrophotometer V-570 (JASCO).

Fluorescent emission: Fluorescent spectra were recorded on Luminescence Spectrometer LS 50B (PerkinElmer Inc., Waltham, MA, USA) with an excitation wavelength of 497 nm.

Longitudinal Relaxation time (T_1): The proton longitudinal relaxation times (T_1) of modified LDL were measured at room temperature (25 °C) on an mq60 Minispec NMR Analyzer (Bruker) at field strength of 60 MHz. An aliquot (200 μL) of each sample was used for measurement.

ICP-MS analysis: The ICP-MS analyses were carried out in Pennsylvania Animal Diagnostic Laboratory System in New Bolton Center (Kennett Square, PA, USA).

2-4. Characterization of modified LDL with fluorescein-OA (3).

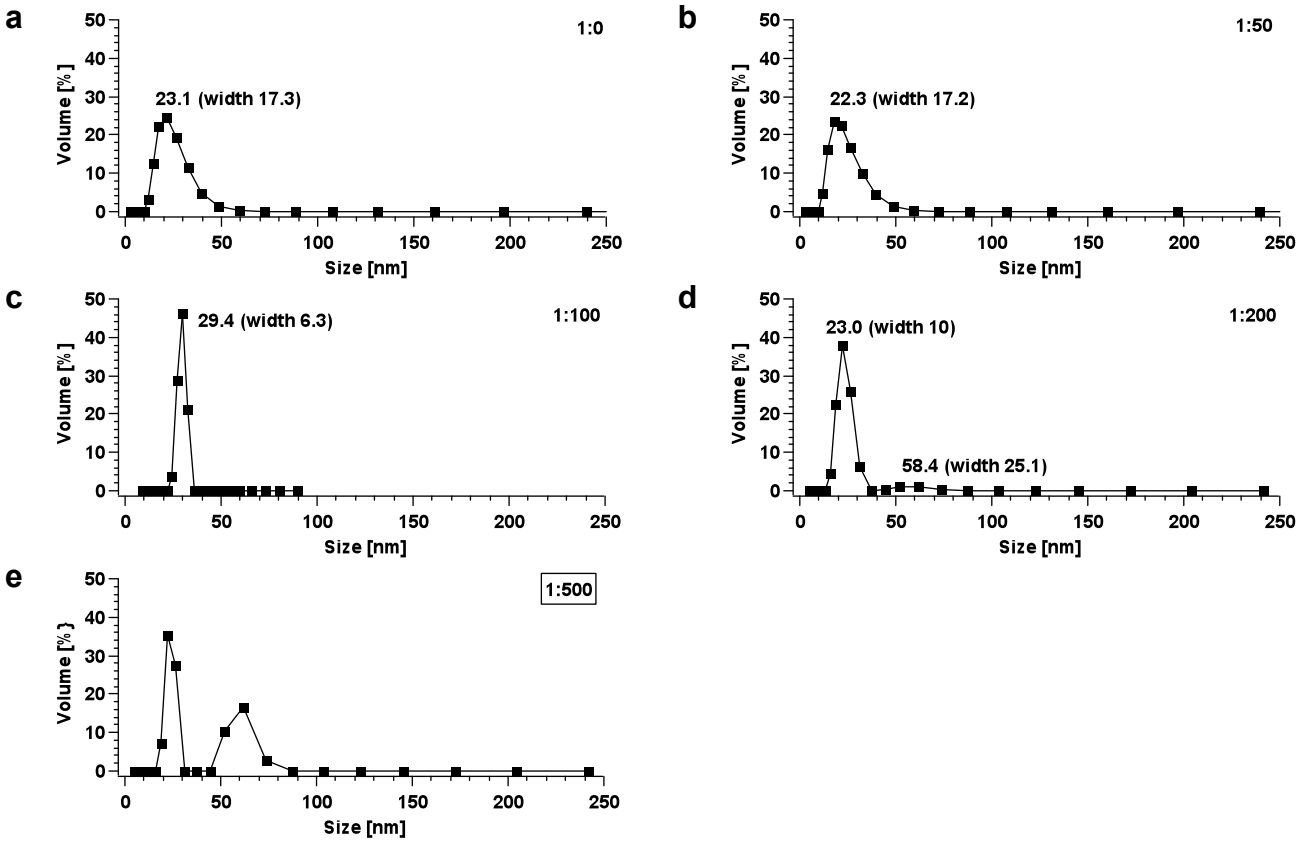


Figure S19. Light scattering analysis of LDL particles with fluorescein-OA **3**. (a) LDL nanoparticle dialyzed (mean = 23.1 nm), (b)-(e) incubated with fluorescein-OA **3** in the molecular ratios of (b) 1:50 (mean = 22.3 nm), (c) 1:100 (mean = 29.4 nm), (d) 1:200 (mean = 23.0 nm (97.4%), mean = 58.4 nm (2.6%)). The values in Y axis are relative volumes (%).

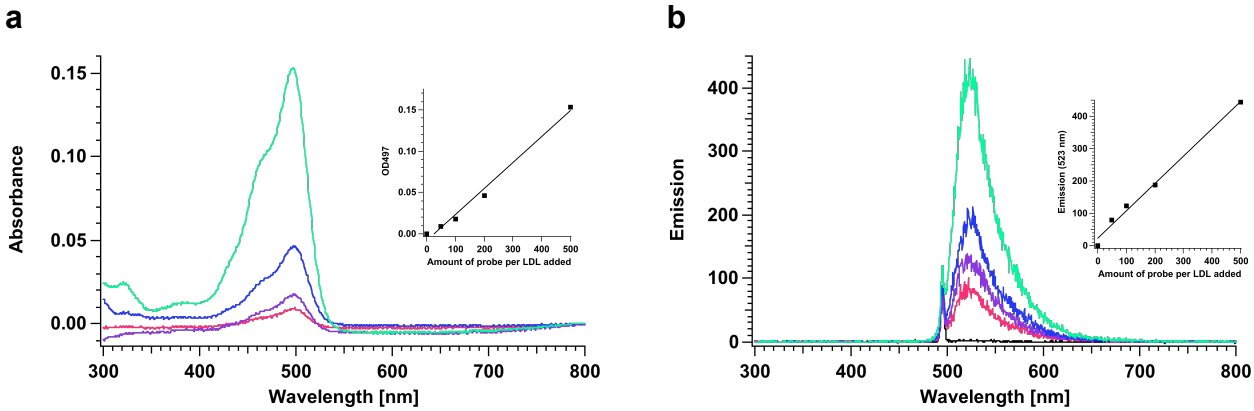


Figure S20. UV-Vis absorption spectra (a) and fluorescent spectra (b) of LDL particles modified with fluorescein-OA **3**. Molecular ratio of LDL to fluorescein-OA **3** are 1:0 (black line), 1:50 (pink lines), 1:100 (purple lines), 1:200 (blue lines) and 1:500 (green lines). Self-quenching of fluorescent dyes were not observed in this range.

2-5. Characterization of modified LDL with MRI-CA probe GdDO3A-OA Gd2.

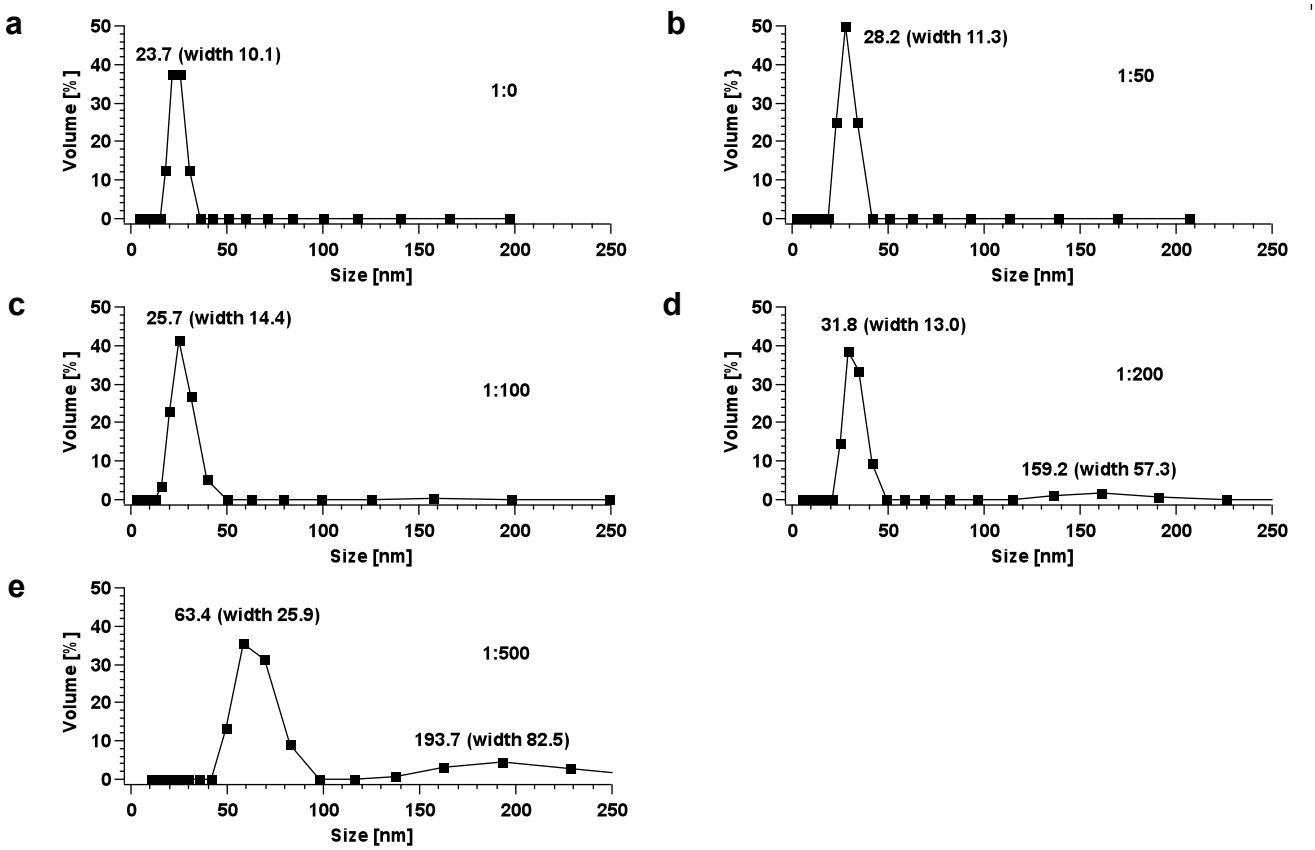


Figure S21. Light scattering analysis of LDL particles modified with GdDO3A-OA Gd**2**. (a) LDL nanoparticle dialyzed, (b) incubated with Gd**2** in the molecular ratio of 1:50, (c) 1:100, (d) 1:200 (ratio of peak areas are 96.3%, (mean= 31.8 nm) and 3.7% (mean= 159.2 nm)), (d)(f) 1:500, (ratio of peak areas are 88.8% (mean= 63.4 nm) and 11.2% (mean= 193.7 nm)). The values in Y axis is relative volumes (%).

Table S1. T_1 relaxation time constant of modified LDL particles with GdDO3A-OA (Gd**2**) at 60 MHz and 25 °C.*

Starting Ratio of LDL to GdDO3A-OA	PBS 0.0067 M	1:0 (neat LDL)	1:50	1:100	1:200	1:500
T_1 [ms]	4030	3290	658	550	192	131
1/ T_1 [s^{-1}]	0.248	0.304	1.520	1.818	5.208	7.634

*Concentration of G^{3+} and LDL were not defined in these measurements.

3. *In vivo* MRI study using mouse model of atherosclerosis

3-1. Animal model of atherosclerosis

Genetically-modified mouse models including $LDLr^{-/-}$ and $ApoE^{-/-}$ are routinely used in the research of atherosclerosis since the atheroplaques in these models exhibit certain characteristics of human atheroplaques. Since mice are naturally resistant to atherosclerosis, feeding the gene knockout mice with a high fat die for 3-4 months is usually necessary to induce extensive plaque formation in the walls of major arteries.

3-2. In vivo injection of GdDO3A-LDL versus ProHance® to LDLr$^{-/-}$ and ApoE$^{-/-}$

Table S2 The accumulation of Gd^{3+} in the aorta of atherosclerosis mouse models receiving Gd**2**-loaded LDL *versus* ProHance®

Tracer injected	mouse model	Dose of Gd [μmol Gd/kg]	Dry weight of tissue (mg)	Gd^{3+} content by ICP-MS [ppm]
GdDO3D-LDL	apoE$^{-/-}$	0.16	8.3	0.073 (n=1)
GdDO3D-LDL	LDL$^{-/-}$	4.67	1.3± 0.15	0.20 ± 0.03 (n=4)
Prohance®	apoE$^{-/-}$	11.6	5.1	< 0.01 (n=1)**
Prohance®	LDL$^{-/-}$	11.0	1.7± 0.19	< 0.01 (n=4)**

* Calculated based on the ICP-MS result of labeled LDL.
** 0.01 ppm is the detection threshold.

3-3. In vivo MRI measurement

In vivo MRI was performed with Varian DirectDrive console (Varian Inc., Palo Alto, CA, USA) interfaced with a horizontal bore (ID = 33 cm) 9.4 T magnet equipped with a gradient coil (ID = 12 cm) capable of generating magnetic field gradient up to 40 gauss/cm. A quadrature volume ^{1}H coil (ID = 3.5 cm, length = 8 cm) was used to house the mouse and acquire MR imaging signal. A tail vein catheter was placed in the mouse, which was sedated by isoflurane (1% mixed with oxygen) during imaging. ECG and respiration were monitored (SA Instruments, Stony Brook, NY, USA) and the core temperature was maintained at 36.5 ± 0.2°C by warm air.

Following parameters was used for BB images: FOV = 26 x 26 mm^2, matrix size = 256 x 192, slice thickness = 0.5 mm, TR = 1000 ms, TE = 10.4 ms, echo train length = 1. For WB images, same parameters were used except for TR = 1 heart beat (about 120 ms), TE = 2.4 ms.

Three slices parallel to the red line were identified on a scout image (Fig. 4a in manuscript) that encompassed the ascending and descending aorta. Image acquisition was ECG and respiration double gated. A multi-slice gradient echo sequence (TR = 1 heart beat, about 120 ms, TE = 2.4 ms) was used to acquire a 'white blood' (WB) image that was used to identify blood vessels and distinguish them from other anatomical features such as the trachea (Fig. 4b in manuscript). In order to idenify plaques within the wall of blood vessel, 'black blood' (BB) images were obtained in which the signal from blood is suppressed. BB images were acquired using a multi-slice fast spin echo sequence (TR = 1000 ms, TE = 10.4 ms, echo train length = 1) before injection (pre-scan, Fig. 4c in manuscript) and at 30, 100, and 220 minutes post-injection (Fig. 4d-f in manuscript).

3-4. Quantification of MRI enhancement

We have quantified the enhancement using a formula by van Bochoveet al.[3] (ref. Contrast Media Mol. Imaging, 2011, *6*, 35-45). Briefly normalized percentage signal enhancement (%NSE) was determined according to

$$\%NSE = \left(\frac{Iwall/Iref}{Iwallpre/Irefpre} - 1 \right) x\ 100\%$$

[3] G. S.van Bochove, L. M. E. Paulis, D. Segers, W. J. M. Mudler, R. Krams, K. Nicolay and G. J. Strijkers, *Contrast Media Mol. Imaging,* 2011, **6**, 35.

I_{wall} and I_{ref} are intensity measured at time point s after contrast agent injection from the region of interest placed on the vessel wall and reference phantom, respectively. $I_{wallpre}$ and I_{refpre} are the respective intensity from wall and reference before contrast injection (pre).

From pre-contrast image, and 30, 100 and 220 min post contrast images, %NSE of 0%, 61%, 82% and 45% were obtained, respectively. The highest %NSE was achieved 100 min post injection.