Supplementary Information

Synthesis and in Vitro Evaluation of Biotinylated Dextran-Derived Probe for Molecular Imaging

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EXPERIMENTAL SECTION

Chemicals. All solvents used were laboratory grade and were dried over the appropriate drying agents if required. All reagents were used as supplied by commercial sources, unless otherwise stated. Water refers to high purity water obtained from the "PuriteSTILL Plus" purification system, with conductivity of 0.04 μS cm^{-1}. Reactions requiring anhydrous conditions were carried out using Schlenk-line techniques under an atmosphere of dry argon.

Chromatography. Flash column chromatography was performed using flash silica gel 60 (70-230 mesh) from Merck. Thin layer chromatography (TLC) was performed on aluminum sheet silica gel plates with 0.2 mm thick silica gel 60 F254 (E. Merck) using different mobile phases. The compounds were visualized by UV irradiation (254 nm) or iodine staining.

Reverse Phase-High Pressure Liquid Chromatography (RP-HPLC) was performed at room temperature on a Varian PrepStar Instrument, Australia, equipped with PrepStar SD-1 pump heads. UV absorbance was measured using a ProStar 335 photodiode array detector at 254 nm. Analytical RP-HPLC was performed in a stainless steel Chromsep (length 250 mm, internal diameter 4.6 mm, outside diameter 3/8 inch and particle size 8 μm) C_{18} column and semi-preparative RP-HPLC was performed on a stainless steel Chromsep (length 250 mm, internal diameter 21.2 mm and particle size 5 μm) C_{18} column (Varian, Advanced Chromatographic Solutions). The compounds (**8** and **L**) were purified using *Method A*: gradient with the mobile phase starting from 95% solvent A (H_2O, 0.1% TFA) and 5% of solvent B (acetonitrile, 0.1% TFA) to 70% B in 10 min, 90% B in 18min, 90% B isocratic till 24 min and decreased to 5% B

in 28 min. The flow rate used for analytical HPLC was 1 mL/min and for semi-preparative HPLC was 15 mL/min.

To check the purity of final complexes (**[Gd.L]** and **[Gd.L]-dex$_{3000}$**), analytical RP-HPLC was carried out on a Perkin Elmer system at 295 K using a 150 x 4.66 mm 4 μ Phenomenex Synergi Fusion-RP 80i column using *Method B*: 95% solvent A (H_2O, 0.1% HCOOH) and 5% solvent B (MeCN, 0.1% HCOOH) isochratic for 2 min, 5% B to 100% solvent B in 15 min and then running isochratic for 1 min and then back to 5% solvent B in the next 2 min. All the solvents for HPLC were filtered through a nylon-66 Millipore filter (0.45μm) prior to use.

Spectroscopy. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker DRX300 spectrometer at room temperature (23°C). The ^{1}H NMR chemical shifts were adjusted to the residual protons of the solvent peaks which were referenced to TMS (0.00 ppm), and ^{13}C NMR chemical shifts were referenced to $CDCl_3$ (77.0 ppm).

Electrospray mass spectra (ESI-MS) were recorded on a SL 1100 system (Agilent, Germany) with ion-trap detection in positive and negative ion mode. High Resolution mass spectra were measured on a Thermo Finnigan LQT.

IR spectra were recorded with a Nicolet Impact 400hD spectrometer using neat compounds as disks with KBr and only the major bands were noted.

Inductively coupled plasma optical emission spectrometry (ICP-OES) for [Gd] analyses was performed using a Jobin–Yvon Ultima 2 spectrometer.

Relaxivity Measurements. Relaxivity measurements were carried out at 37°C, 60 MHz (1.4 T) on a Bruker Minispec mq60 instrument. To measure the changes in longitudinal relaxivity (r_{1p}), MRI experiments were performed without and with increasing concentrations of avidin proportional to a constant concentration of **[Gd.L]** (0.27 mM) and **[Gd.L]-Dex$_{3000}$** (0.2 mM) respectively. The stock solution of avidin (1 mM) was prepared in PBS at pH 7.4. The mean value of three separate measurements was recorded and averaged. The relaxivities of the compounds were calculated as the slope of the function shown in equation (1),

$$1/T_{1,obs} = 1/T_{1,d} + r_1 \times [\mathrm{GdL}] \quad (1)$$

where $T_{1,obs}$ is the measured T_1, $T_{1,d}$ is the diamagnetic contribution of the solvent (calculated to be 4000 ms) and [GdL$_n$] is the concentration in mM of the appropriate Gd(III) complex. Error for all relaxivity values was less then 0.06 $mM^{-1}s^{-1}$.

Synthesis of Ligands and Complexes.

(*S*)-methyl 13,13-dimethyl-3,11-dioxo-1-phenyl-2,12-dioxa-4,10-diazatetradecane-5-carboxylate (1). A solution of (*S*)-13,13-dimethyl-3,11-dioxo-1-phenyl-2,12-dioxa-4,10-diazatetradecane-5-carboxylic acid (3 g, 5.3 mmol), MeOH (0.25 g, 7.9 mmol), NMM (1.06 g, 10.5 mmol) and HOBt (0.85 g, 6.3 mmol) in anhydrous DMF (10 mL) was stirred at 0-5˚C for 15 min and then EDC.HCl (1.21 g, 6.3 mmol) was added. The reaction mixture was stirred for 3 h at room temperature. The completion of reaction was verified by TLC, the reaction mixture poured into water (40 mL) and extracted with EtOAc (3x50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate evaporated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 10% EtOAc in Hexane, R_f = 0.6) to give **1** as a transparent gum (1.94 g, 94%). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 1.22 -

1.43 (m, 4H, CH_2); 1.35 (s, 9H, CH_3); 1.53 - 1.81 (m, 2H, CH_2); 2.95-3.10 (m, 2H); 3.63 (s, 3H, CH_3); 4.15-4.32 (m, 1H, CH); 4.86 (br. s, 1H, NH); 5.02 (s, 2H, OCH_2); 5.76 (br. S, 1H, NH); 7.18 - 7.29 (m, 5H, CH_{Ar}). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 22.1; 28.0, 29.1; 31.5; 39.6; 51.8; 53.4; 66.4; 78.5; 127.7; 128.1; 136.0; 155.8; 172.6. ESI-HRMS (+): calcd $C_{20}H_{30}NaN_2O_6$: m/z 417.2002 $(M+Na)^+$; found 417.2003 $(M+Na)^+$.

(*S*)-methyl 2-amino-6-(*tert*-butoxycarbonylamino)hexanoate (2). A solution of compound **1** (1.9 g, 4.8 mmol), (10%) Pd-C (0.4 g, w/w) and H_2 (40 psi) in MeOH (10 mL) was stirred at room temperature in a Parr apparatus for 6 h. The reaction mixture was filtered through Celite, the filtrate evaporated under reduced pressure to give **2** as a transparent gum (1.1 g, 88%). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 1.36 - 1.53 (m, 4H, CH_2); 1.42 (s, 9H, CH_3); 1.60-1.82 (br. m, 2H, CH_2); 2.25 (br. s, 2H, NH_2); 3.10 (q, *J*=6 Hz, 2H, CH_2); 3.42-3.53 (m, 1H, CH); 3.71 (s, 3H, CH_3); 4.86 (br. s, 1H, NH). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 22.7; 28.3; 29.7; 34.1; 40.2; 51.9; 54.1; 78.9; 155.9; 175.9. ESI-HRMS (+): calcd $C_{12}H_{24}N_2O_4$: m/z 261.1814 $(M+H)^+$; found 261.2 $(M+H)^+$.

(*S*)-methyl 6-(*tert*-butoxycarbonylamino)-2-(5-((3a*R*,4*R*,6a*S*)-2-oxo-hexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexanoate (3). A solution of compound **2** (1 g, 3.8 mmol), D-biotin (1.03 g, 4.2 mmol), NMM (0.77 g, 7.6 mmol) and HOBt (0.57 g, 4.2 mmol) in anhydrous DMF (5 mL) was stirred at 0-5˚C for 15 min and then EDC.HCl (0.81 g, 4.2 mmol) was added. The reaction mixture was stirred overnight at room temperature. The completion of reaction was verified by TLC, solution poured into water (50 mL) and extracted with EtOAc (3x50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography

(silica gel, 5% MeOH in CH_2Cl_2, R_f = 0.4) to give **3** as a yellow gum (1.27 g, 68%). ^{1}H NMR ($CDCl_3$, 300 MHz), δ (ppm): 1.25 - 1.55 (br. m, 6H, C*H*$_2$); 1.43 (s, 9H, C*H*$_3$); 1.57-1.91 (br. m, 6H, C*H*$_2$); 2.25 (t, *J*=7 Hz, 2H, CH_2CONH); 2.64 – 2.77 (m, 1H, SC*H*$_2$); 2.81 – 2.96 (m, 1H, SC*H*$_2$); 2.97 – 3.20 (m, 3H, NHC*H*$_2$, SC*H*); 3.71 (s, 3H, CH_3); 4.25 – 4.36 (m, 1H, C*H*NHCO); 4.44 – 4.58 (m, 2H, C*H*NHCO, COC*H*NHCO); 4.98 (br. s, 1H, NH);); 6.44 (s, 1H, NH);); 6.82 (s, 1H, NH);); 7.46 (br. s, 1H, NH). ^{13}C NMR ($CDCl_3$, 75 MHz), δ (ppm): 22.5; 25.1; 27.7; 28.1; 29.2; 31.1; 35.1; 39.8; 40.1; 51.7; 51.9; 53.2; 55.7; 60.0; 61.6; 78.6; 155.8; 164.3; 173.3; 173.6. ESI-HRMS (+): calcd $C_{22}H_{38}NaN_4O_6S$: m/z 509.2410 $(M+Na)^+$; found 509.2416 $(M+Na)^+$.

(*S*)-6-(*tert*-butoxycarbonylamino)-2-(5-((3a*R*,4*R*,6a*S*)-2-oxo-hexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexanoic acid (4). A solution of compound **3** (1.1 g, 2.3 mmol) in THF:MeOH:water (3:2:2, 7 mL) was stirred at 0-5˚C for 15 min and then LiOH (0.08 g, 3.4 mmol) was added. The reaction mixture was stirred for 3 h at room temperature. The progress of the reaction was monitored by ESI-MS. After completion, the pH was adjusted by 1 N HCl. The solvent was evaporated under reduced pressure to afford **4** as an off-white solid (1.15 g). ^{1}H NMR (D_2O, 300 MHz), δ (ppm): 1.24 - 1.50 (br. m, 6H, C*H*$_2$); 1.39 (s, 9H, C*H*$_3$); 1.52-1.86 (br. m, 6H, C*H*$_2$); 2.28 (t, *J*=7 Hz, 2H, C*H*$_2$CONH); 2.70 – 2.79 (m, 1H, SC*H*$_2$); 2.90 – 2.98 (m, 1H, SC*H*$_2$); 2.99 – 3.07 (m, 2H, NHC*H*$_2$); 3.24 – 3.36 (m, 1H, SC*H*); 4.07 – 4.16 (m, 1H, C*H*NHCO); 4.33 – 4.44 (m, 1H, C*H*NHCONH); 4.51 – 4.63 (m, 1H, C*H*NHCONH). ^{13}C NMR (D_2O, 75 MHz), δ (ppm): 22.6; 25.1; 27.6; 27.7; 27.8; 28.5; 31.3; 35.4; 39.7; 39.8; 54.9; 55.3; 60.2; 62.0; 80.7; 158.2; 165.2; 176.0; 179.3. ESI-HRMS (+): calcd $C_{21}H_{36}NaN_4O_6S$: m/z 495.2253 $(M+H)^+$; found 495.2260 $(M+H)^+$.

***trt*-butyl (*S*)-6-(4-nitrobenzylamino)-6-oxo-5-(5-((3a*R*,4*R*,6a*S*)-2-oxo-hexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexylcarbamate (5).** A solution of compound **4** (1 g, 2.1 mmol), (4-nitrophenyl)methanamine (0.48 g, 3.2 mmol), NMM (0.43 g, 4.2 mmol) and HOBt (0.32 g, 2.3 mmol) in anhydrous DMF (5 mL) was stirred at 0-5˚C for 15 min and then EDC.HCl (0.45 g, 2.3 mmol) was added. The reaction mixture was stirred for 6 h at room temperature. The completion of reaction was verified by TLC, solution poured into water (50 mL) and extracted with EtOAc (3x50 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate evaporated under reduced pressure. The residue was purified by column chromatography (silica gel, 5% MeOH in CH_2Cl_2, R_f = 0.35) to give **5** as a yellow gum (0.98 g, 76%). ^{1}H NMR (DMSO-d_6, 300 MHz), δ (ppm): 1.14 - 1.41 (br. m, 6H, CH_2); 1.36 (s, 9H, CH_3); 1.42-1.76 (br. m, 6H, CH_2); 2.14 (t, *J*=7 Hz, 2H, CH_2CONH); 2.52 – 2.62 (m, 1H, SCH_2); 2.75 – 2.82 (m, 1H, SCH_2); 2.83 – 2.93 (m, 2H, NHCH_2); 3.01 – 3.12 (m, 1H, SC*H*); 4.04 – 4.15 (m, 1H, C*H*NHCO); 4.16 – 4.24 (m, 1H, C*H*NHCONH); 4.25 – 4.33 (m, 1H, C*H*NHCONH); 4.39 (s, 2H, CH_2Ar); 6.34 (s, 1H, N*H*); 6.39 (s, 1H, N*H*); 6.74 (br. s, 1H, N*H*); 7.49 (d, *J*=8.5 Hz, 2H, H_{Ar}); 7.96 (s, 1H, N*H*); 8.17 (d, *J*=8.5 Hz, 2H, H_{Ar}); 8.58 (s, 1H, N*H*). ^{13}C NMR (DMSO-d_6, 75 MHz), δ (ppm): 21.7; 24.1; 26.9; 27.0; 27.2; 28.1; 30.4; 33.8; 40.5; 41.2; 47.5; 51.6; 54.3; 58.1; 59.9; 76.2; 122.3; 126.9; 145.3; 146.6; 154.4; 161.6; 171.2; 173.4. ESI-HRMS (+): calcd $C_{28}H_{42}NaN_6O_7S$: m/z 629.2733 $(M+Na)^+$; found 629.2735 $(M+Na)^+$.

(*S*)-6-amino-*N*-(4-nitrobenzyl)-2-(5-((3a*R*,4*R*,6a*S*)-2-oxo-hexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexanamide (6). A solution of compound **5** (0.9 g, 1.5 mmol) was dissolved in CH_2Cl_2 (9 mL), TFA (1 mL) was added and the mixture stirred at room temperature for 2 h. After completion, the reaction mixture was evaporated, dissolved in CH_2Cl_2

(10 ml) and extracted with saturated aqueous Na_2CO_3 solution (2x10 mL). The organic layer was evaporated under reduced pressure to give **6** as a yellow gum (0.7 g, 84%)**.** ^{1}H NMR (DMSO-d_6, 300 MHz), δ (ppm): 1.12 - 1.41 (br. m, 4H, CH_2); 1.42-1.81 (br. m, 8H, CH_2); 2.16 (t, *J*=7 Hz, 2H, CH_2CONH); 2.53 – 2.65 (m, 1H, SCH_2); 2.69 – 2.87 (m, 3H, SCH_2, NHCH_2); 3.01 – 3.12 (m, 1H, SC*H*); 4.06 – 4.16 (m, 1H, C*H*NHCO); 4.16 – 4.35 (m, 2H, C*H*NHCONH); 4.40 (s, 2H, CH_2Ar); 6.39 (s, 1H, N*H*); 6.42 (s, 1H, N*H*); 7.49 (d, *J*=9 Hz, 2H, H_{Ar}); 7.86 (br. s, 2H, NH_2); 8.05 (s, 1H, N*H*); 8.17 (d, *J*=9 Hz, 2H, H_{Ar}); 8.62 (br. s, 1H, N*H*). ^{13}C NMR (DMSO-d_6, 75 MHz), δ (ppm): 25.1; 27.8; 29.2; 30.6; 30.8; 33.8; 37.5; 41.1; 44.2; 44.3; 55.2; 58.1; 61.8; 63.7; 126.0; 130.6; 149.0; 150.3; 165.4; 174.9; 175.1. ESI-HRMS (+): calcd $C_{23}H_{34}N_6O_5S$: m/z 507.2390 $(M+H)^+$; found 507.2371 $(M+H)^+$.

[4,7-Bis-carboxymethyl-10-(2-((*S*)-6-(4-nitrobenzylamino)-6-oxo-5-(5-((3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexylamino)-2-oxoethyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid (8). A solution of compound **6** (0.32 g, 0.63 mmol), tris-*tert*-butyl DOTA (0.3 g, 0.52 mmol), NMM (0.11 g, 1.05 mmol) and HOBt (0.08 g, 0.59 mmol) in anhydrous DMF (2 mL) was stirred at 0-5˚C for 15 min and then EDC.HCl (0.11 g, 0.59 mmol) was added. The reaction mixture was stirred overnight at room temperature. The completion of intermediate (tris-*tert*-butyl ester **7**) formation was verified by ESI-LRMS [ESI-LRMS (+): calcd $C_{51}H_{84}N_{10}O_{12}S$: m/z 1060.5 $(M+H)^+$; found 1060.7 $(M+H)^+$]. Reaction mixture was concentrated at reduced pressure and the crude intermediate was subjected to *tert*-butyl deprotection using neat TFA (10 mL). After TFA evaporation, the crude residue was re-dissolved in CH_2Cl_2 and concentrated three times and then the procedure was repeated with methanol to completely evaporate TFA. The residue was purified by preparative reverse phase

HPLC (method A, t_R = 3.8 min). After lyophilization, **8** as a light yellow sticky solid was obtained (0.1 g, 40%). ^{1}H NMR (D_2O, 300 MHz), δ (ppm): 1.12 - 1.83 (br. m, 12H, C*H*$_2$); 2.24 (t, *J*=6.5 Hz, 2H, C*H*$_2$CONH); 2.57 – 2.71 (m, 1H, SC*H*$_2$); 2.75 – 2.88 (m, 1H, SC*H*$_2$); 2.90 – 3.72 (br. m, 27H, C*H*$_{2(ring)}$, C*H*$_2$CO, SC*H*, NHC*H*$_2$); 4.12 – 4.34 (m, 2H, C*H*NHCO, C*H*NHCONH); 4.37 – 4.51 (m, 1H, C*H*NHCONH); 4.43 (s, 2H, C*H*$_2$Ar); 7.38 (d, *J*=8 Hz, 2H, *H*$_{Ar}$); 8.10 (d, *J*=8 Hz, 2H, *H*$_{Ar}$). ^{13}C NMR (D_2O, 75 MHz), δ (ppm): 22.5; 25.1; 27.6; 30.4; 31.4; 34.9; 36.9; 39.6; 42.3; 53.2; 53.4; 53.6; 53.7; 53.9; 54.1; 54.9; 55.2; 60.0; 60.2; 61.8; 123.8; 127.6; 146.1; 146.7; 165.1; 171.4; 172.8; 174.6; 176.8; 176.9. ESI-LRMS (+): calcd $C_{39}H_{60}N_{10}O_{12}S$: m/z 893.4 $(M+H)^+$; found 893.6 $(M+H)^+$.

[4,7-Bis-carboxymethyl-10-(2-((*S*)-6-(4-isothiocyanatobenzylamino)-6-oxo-5-(5-((3a*S*,4*S*,6a*R*)-2-oxo-hexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanamido)hexylamino)-2-oxoethyl)-1,4,7,10-tetraaza-cyclododec-1-yl]-acetic acid (L). After reduction of the nitro group by hydrogenation (Pd-C (10%), H_2, MeOH, 50 psi), 0.1 g (0.11 mmol) of the product were solved in water (2 mL), stirred at room temperature and thiophosgene (0.03 g, 0.22 mmol) in 1 mL CCl_4 was added dropwise. The pH of the mixture maintained 7.5-8 using 1 M NaOH. The reaction was monitored by ESI-MS. The reaction mixture was evaporated and the residue was purified by preparative HPLC (method A, t_R = 3.5 min). After lyophilization, **L** as a light yellow sticky solid was obtained 0.06 g (64%). ^{1}H NMR (D_2O, 300 MHz), δ (ppm): 1.15 - 1.84 (br. m, 12H, C*H*$_2$); 2.26 (m, 2H, C*H*$_2$CONH); 2.61 – 2.76 (m, 1H, SC*H*$_2$); 2.80 – 2.92 (m, 1H, SC*H*$_2$); 2.95 – 4.10 (br. m, 27H, C*H*$_{2(ring)}$, C*H*$_2$CO, SC*H*, NHC*H*$_2$); 4.17 – 4.37 (m, 2H, C*H*NHCO, C*H*NHCONH); 4.26 (s, 2H, C*H*$_2$Ar); 4.42 – 4.56 (m, 1H, C*H*NHCONH); 7.43 – 7.58 (m, 4H, *H*$_{Ar}$). ^{13}C NMR (D_2O, 75 MHz), δ (ppm): 22.6; 25.0; 27.6; 29.4; 30.5; 35.0; 38.2; 42.3; 44.4;

48.3; 48.9; 50.7; 52.6; 54.1; 54.2; 55.1; 55.2; 59.1; 60.2; 61.8; 126.9; 128.2; 130.2; 135.0; 137.9; 165.1; 171.9; 174.2; 176.7; 179.0; 179.4. ESI-LRMS (+): calcd $C_{40}H_{60}N_{10}O_{10}S_2$: m/z 905.4 $(M+H)^+$; found 905.2 $(M+H)^+$.

Preparation of the gadolinium complex of L. Gadolinium complex of **L** was prepared from mixing solutions of the ligand (1 eq) and $GdCl_3.6H_2O$ (1.1 eq) in water. The reaction mixture was stirred at 60° C for 20 h. The pH was periodically checked and adjusted to 6.5 using solutions of NaOH (1 M) and HCl (1 N) as needed. After completion, the reaction mixture was cooled down and passed through Chelex-100 to trap free Gd^{3+} ions, and the Gd^{3+}-loaded complexes were eluted. The fractions were dialyzed (500 M.Wt cutoff; Spectra/Pro® biotech cellulose ester dialysis membrane, Spectrum Laboratories) and lyophilized to obtain off-white solids. The absence of free Gd^{3+} was checked with xylenol orange indicator. These complexes were characterized by ESI-LRMS in positive mode and the appropriate isotope pattern distribution for Gd^{3+} were recorded. The purity of complex was analysed by analytical reverse phase HPLC (method B). **[Gd.L**] ESI-LRMS (+): calcd $C_{40}H_{57}{}^{155}GdN_{10}O_{10}S_2$: m/z 1056.2 $[M-H]^-$; found 1056.1 $[M-H]^-$, HPLC t_R = 13.6 min, r_{1p} = 5.29 $mM^{-1}s^{-1}$ (60 MHz, 310K).

Preparation of [Gd.L]-dex$_{3000}$. A solution of **[Gd.L]** (0.02 g, 0.03 mmol) in water (5 mL) was stirred at room temperature and dextran (MWt 3000, 0.04 g, 0.02 mmol) was added. The pH of the mixture was maintained to 8.5 using 1 M NaOH and 1 N HCl. The reaction was stirred overnight at room temperature. To remove excess **[Gd.L]** from the reaction mixture, the solution was dialysed using Omega 3K PES ultrafiltration membrane with cut off 3000 Da and lyophilized to obtain off-white solids. The purity of complex was analysed by analytical reverse phase HPLC (method B; t_R = 11.9 min). **[Gd.L]-dex$_{3000}$**: r_{1p} = 7.73 $mM^{-1}s^{-1}$ (60 MHz, 310K). IR:

721.9 (aromatic C-H); 1013.9 (C-O); 1392.4 (C-H); 1413.2 (C=S); 1473.6 (NCN); 1618.0 (C=O, carboxylate); 1681.8 (C=O, amide); 3298.1 (OH).

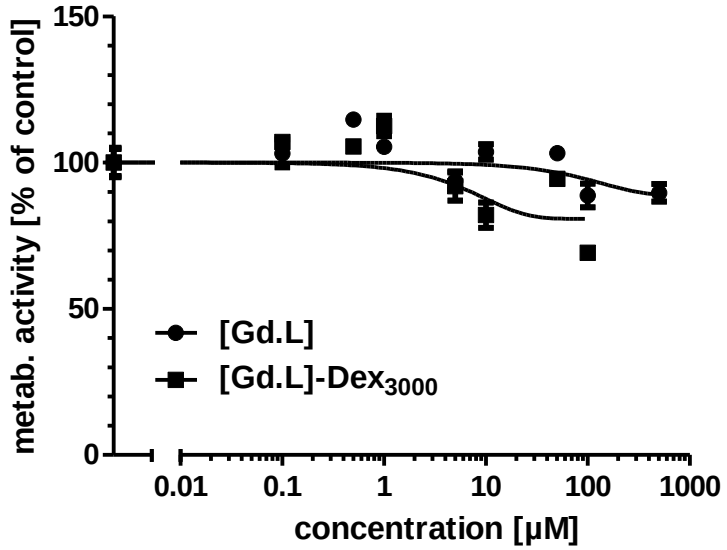
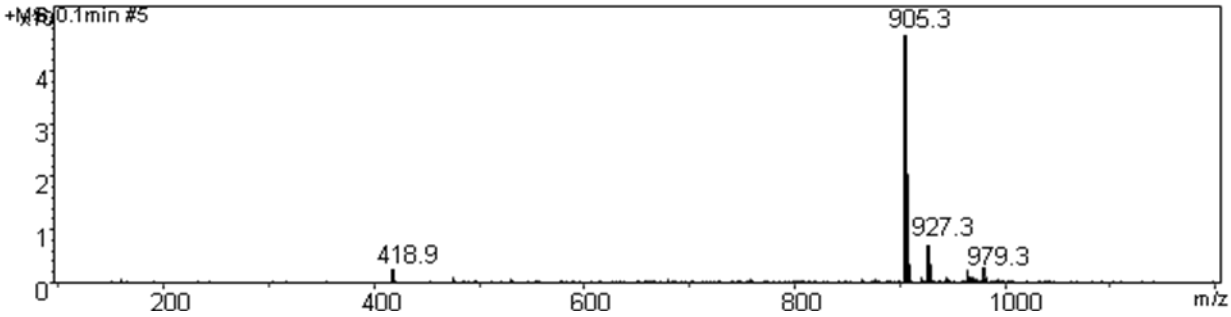


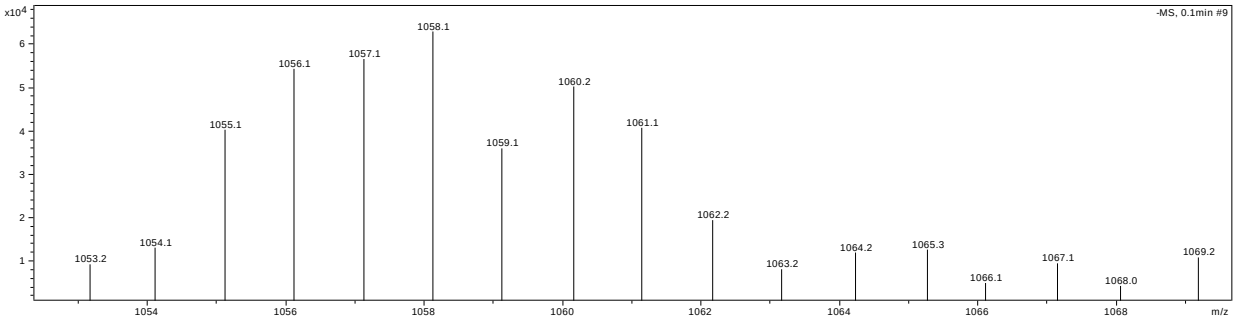
Figure S1. Acute cytotoxicity of **[Gd.L]** and **[Gd.L]-Dex$_{3000}$**

Metabolic activity as a measure of viability was determined by an XTT-based assay. Cells were incubated with **[Gd.L]** and **[Gd.L]-Dex$_{3000}$** for 5h in HBSS/10 mM HEPES as described in the manuscript.

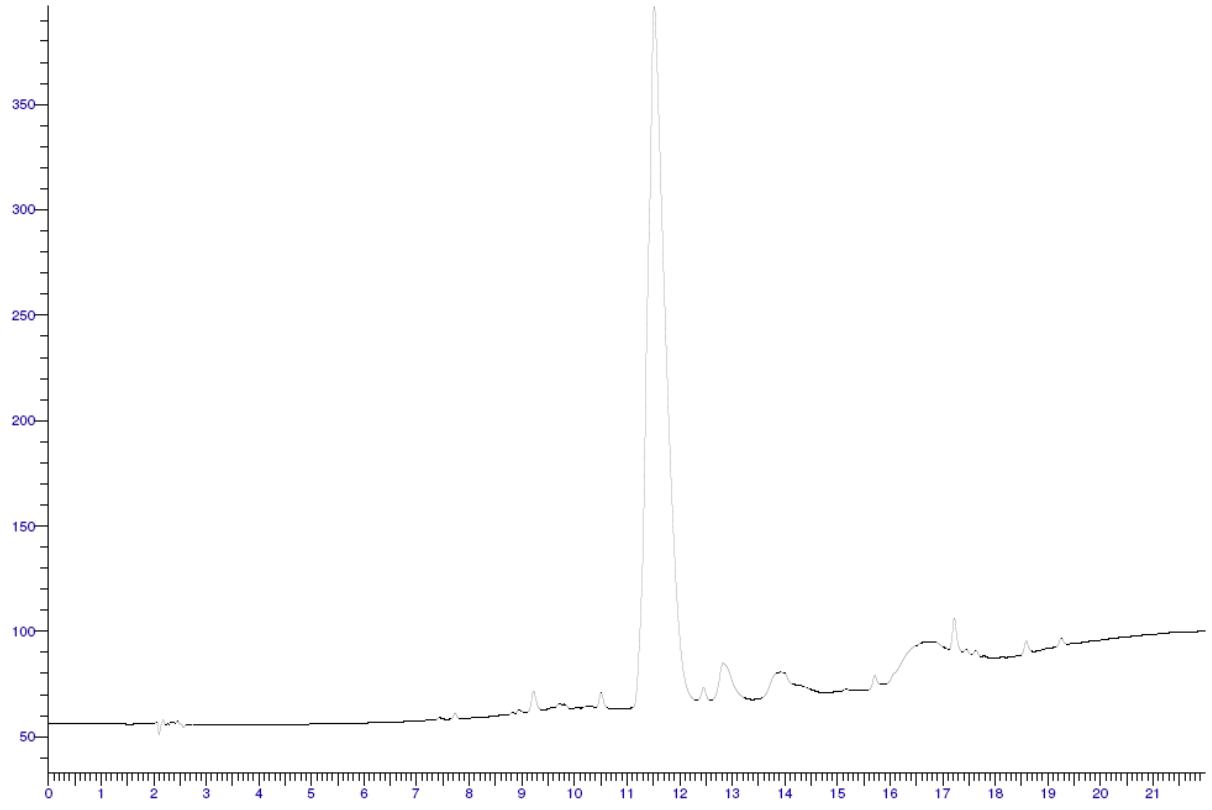
ESI-MS of L (positive mode)



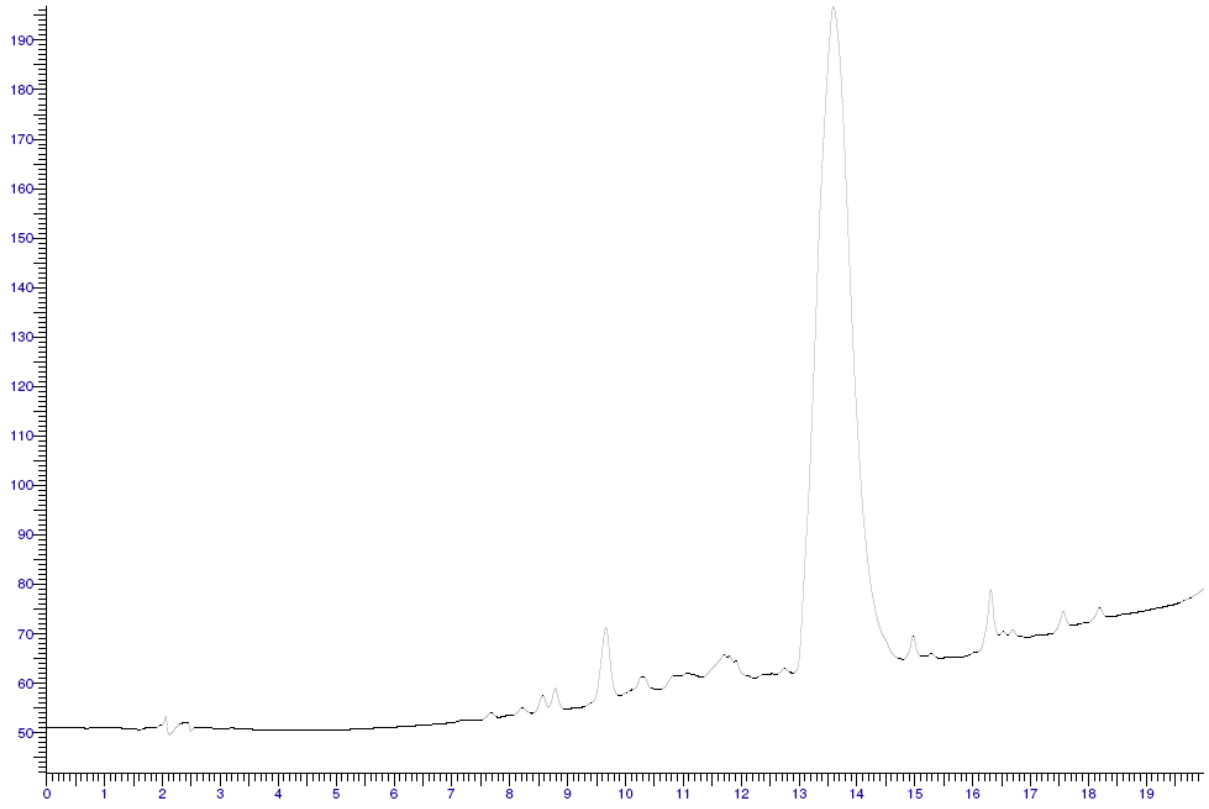
ESI-MS of [Gd.L] (negative mode)



HPLC chromatogram of [Gd.L]



HPLC chromatogram of [Gd.L]-Dex_{3000}



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