Supporting Information

A Bimodal Fluorescence-Magnetic Resonance Contrast Agent for Apoptosis Imaging

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Materials and general method

All chemicals used for synthesis were purchased from commercial sources such as Sigma-Aldrich, Oakwood Products, Alfa Aesar, Chem-Impex Int'l. Inc., Ark Pharmacies, Inc. and were used as received. Flash chromatography was performed using 230-400 mesh silica gel P60 (SiliCycle Inc). Solvents were purchased from Sigma-Aldrich and used without further purifications. Anhydrous solvents were obtained from the solvent system. Deuterated solvents were purchased from Sigma-Aldrich. The following abbreviations are used to describe various chemicals: dichloromethane (DCM), acetonitrile (ACN), tetrahydrofuran (THF), trifluoroacetic acid (TFA), dimethylformamide (DMF), *p*-toluenesulfonic acid monohydrate (PTSA). Recombinant human caspase-3, caspase-7, cathepsin B were purchased from R&D Systems; human lysosome was purchased from Sigma-Aldrich. Appropriate storage buffers and assay buffers were prepared according to the protocols from the manufacturers. All buffers were prepared in Milli-Q® water and brought to the appropriate pH with aqueous HCl or NaOH, and

were filtered through microfilter (0.2 μm) prior to use. All buffers for the enzymatic assays were made fresh and used within a week. We noticed that the caspase-3/7 assay buffer will degrade even when stored at -20 °C.

Table S1. Enzyme buffers used in this study

Enzyme Buffer	Components	pH
Caspase-3 & 7 storage	25 mM HEPES, 200 mM NaCl, 10 mM dithiothreitol (DTT), 10% (w/v) sucrose	7.5
Caspase-3 & 7 assay	25 mM HEPES, 0.1% (w/v) CHAPS, 10 mM dithiothreitol (DTT)	7.5
Cathepsin B storage	25 mM Tris, 150 mM NaCl	8.0
Cathepsin B assay	25 mM MES, 5 mM DTT	5.0
Lysozyme assay	36.5 mM KH_2PO_4, 13.4 mM K_2HPO_4	6.2

NMR and HR-MS Methods

NMR spectra were recorded on the Bruker Avance III 500 MHz system equipped with DCH CryoProbe at 25 °C. Chemical shifts are reported in ppm (δ) and are referenced to residual protic peaks. The following abbreviations are used to describe coupling constants: singlet (s), doublet (d), triplet (t), quartet (q), doublet doublet (dd), doublet triplet (dt), doublet quartet (dq), doublet doublet triplet (ddt), multiplet (m), and broad singlet (bs). High-resolution mass spectra were acquired at NUANCE (Northwestern Atomic and Nanoscale Characterization Experimental Center).

HPLC Methods

Analytical HPLC was carried out with Agilent 1260 Infinity II LC system equipped with a 1260 Infinity II Quaternary Pump, an inline diode array UV-Vis detector and Agilent 6120 Quadrupole LCMS System. Absorbances at 210 nm and 350 nm were monitored. The column used for analytical HPLC was a XBridge™ C18 5 μm reverse phase column (4.6×150 mm)

Semi-preparative HPLC was carried out with Agilent 1260 Infinity II LC system equipped with PrepStar 218 solvent delivery module, an inline diode array UV-Vis detector and 440-LC Fraction Collector. Absorbances at 210 nm and 350 nm were monitored. The column used for semiprep-HPLC was a XBridge™ Prep C18 5 μm OBD™ reverse phase column (19×150 mm)

General Procedure for Enzymatic Assay

For the fluorescence caspase-3 assay, stock solution of CP1 or CCP1 (0.1 mM) in caspase-3 buffer and caspase-3 (0.8 μg/mL) solution were preheated to 37 °C, and 150 μL of each solution were mixed at a 1:1 volume ratio before taking fluorescence measurements using fluorimeter.

For the relaxometry caspase-3 assay, stock solution of CP1 or CCP1 (0.4 mM) in caspase-3 buffer and caspase-3 (0.8 μg/mL) solution were preheated to 37 °C, and 150 uL of each solution were mixed at a 1:1 volume ratio before taking relaxometry measurements.

Fluorimeter and UV-Vis measurement

Steady-state fluorescence emission and excitation spectra were obtained using a Hitachi F-45000 Fluorescence Spectrophotometer. The excitation slit width, emission slit width, and

photomultiplier voltages were 5 nm, 5 nm, and 700 V, respectively. The excitation wavelength was 325 nm, and the emission spectra were collected from 400-600 nm. Relative fluorescence quantum yields of CP1 and Gad-AIE were determined by comparing the area under the emission of the sample with that of quinoline sulfate in 0.5 M H_2SO_4 (Φ = 0.546). UV-Vis spectra were recorded on an Agilent 8453 spectrophotometer.

Cell Culture

HeLa cells were purchased from American Type Culture Collection (Manassas, VA, U.S.A.) and cultured in DMEM media supplemented with 10% FBS. Cells were grown in a humidified incubator operating at 37 °C and 5.0% CO_2 and harvested with 0.25% TrypLE. Cells were allowed to adhere overnight before all experiments.

Cellular toxicity

The cellular toxicity of CP1 was evaluated with MTS assay. HeLa cells were seeded at a density of 5000 cells per well in a 96-well plate. Cells were incubated with CP1 at concentrations ranging from 0 to 200 μM for 24 h. After incubation, the cell media was aspirated off and 50 ìL of CellTiter 96® AQueous One Solution Reagent (Promega, Madison, WI, U.S.A.) was added to each well and the assay was run according to the manufacturer's protocol. Absorption was read on a Synergy H1 Microplate Reader (BioTek, Winooski, VT, U.S.A.).

Cellular uptake

This was done according to previously reported method.[6] Concentration-dependent uptake was determined in HeLa cells plated at a density of 25,000 cells per well in a 24-well plate. CP1 was dissolved in media at concentrations ranging from 0 to 1 mM and incubated with cells for 24 h.

After incubation, cells were washed twice with 0.5 mL DPBS and centrifuged at 500g for 5 min at 4 °C. The media was aspirated off and cells were resuspended in 500 μL fresh media, vortexed briefly and centrifuged at 500g for 5 min at 4 °C. The media was aspirated off again and cells were resuspended in 200 μL media. An aliquot of 20 μL was used for cell counting and 120 μL was used for analysis of Gd(III) content by ICP-MS. For time-dependent uptake, the same procedure was followed except cells were incubated with 200 μM CP1 for 1, 2, 4, 8, or 24 h.

Fluorescence microscopy and image processing

HeLa cells were seeded at a density of 50,000 cells per plate on a 35 mm FluoroDish (World Precision Instruments, Sarasota, FL, U.S.A.). Cells were incubated with 50 μM CP1 (1mL) for 2 hours, then washed with cell media (1 mL) twice, and incubated with media alone or 1 uM STS in media (1 mL) for 1 hr. The cells were then washed with cell media (1 mL) twice, and incubated with AnnexinV-Alexa Fluro 594 in serum-free DMEM (v/v=1:25, 500uL) at room temperature for 15 mins. Prior to imaging, the cells were washed with serum-free DMEM (1 mL×2) and incubated with serum-free DMEM. Imaging was performed on a DeltaVision Core deconvolution microscope with a × 63 objective equipped with a CoolSnap2 CCD camera (GE Healthcare). CP1 was imaged using a DAPI filter (435/48nm). Annexin V Alexa Fluoro 488 was imaged using a 488 filter set (525/50nm). This microscope is housed in the Northwestern University Biological Imaging Facility, generously supported by the Chemistry of Life Processes Institute and the NU Office for Research. All cell images were processed with ImageJ and subjected to the same background subtraction as well as brightness and contrast adjustments.

Relaxometry at 1.41 T

For the relaxivity measurement, a stock solution of each gadolinium complex was made up in caspase-3 assay buffer. These samples were serially diluted four times to give 300 μL of four to five different sample concentrations. The exact Gd(III) in each sample was determined via ICP-MS.

For the enzymatic assay, a stock solution of CP1 or CCP1 (0.4 mM) and the appropriate enzyme (0.8 μg/mL) are preheated to 37 °C, and are mixed at a 1:1 volume ratio before relaxometry measurements.

The T_1 and T_2 relaxation times were measured on a Bruker mq60 NMR analyzer equipped with Minispec V2.51 Rev.00/NT software (Billerica, MA, U.S.A.) operating at 1.41 T (60 MHz) and 37 °C. Relaxation time determination and analysis was performed as previously described.30

MRI of solution and cell phantom at 7 T

High field relaxivity measurements were performed on a Bruker Pharmascan 7T MRI system with a 16 cm bore and 9 cm shielded gradients, running Paravision 6.0.1. (Bruker Biospin Inc, Billerica, MA, USA). Samples were prepared and placed in flame-sealed glass capillary tubes with approximate diameter of 1 mm. The capillary tubes were secured to a larger tube filled with water, positioned in a volume radiofrequency coil with 23mm diameter, and centered in the magnet bore. T_1 relaxation measurements were made using a variable repetition time accelerated spin echo sequence (RARE-VTR) with the following parameters: Variable TR = 150, 250, 500, 750, 1000, 2000, 4000, 6000, 8000, and 10000 ms; TE = 11 ms, field of view = 25 mm × 25 mm, matrix size = 256 × 256, number of axial slices = 5, slice thickness = 1 mm, and 3 averages. T_2

relaxation measurements were made using a multi slice multi echo sequence (MSME) with TR = 4500 ms, TE=10-300 ms with 10 ms spacing, and slice geometry identical to that of the T_1 map.

T_1 and T_2 analysis were performed in Paravision using built-in mono-exponential curve fitting of image intensities in selected regions of interest.

MRI cell pellet preparation

HeLa cells were plated in 2 separate T70 flasks (A and B) At 60-70% confluency, A was incubated with 7 mL media, while B was treated with 0.2 mM CP1 in 7 mL media. After 24 hrs incubation, cells in each flask were washed twice with 5 mL DPBS, typsinized, transferred to 2 separate falcon tubes and centrifuged at 500g for 5 min at 4 °C. The media was aspirated off and cells were resuspended in 1 mL fresh media and vortexed briefly to mix. For each cell suspension, an aliquot of 20 µL was used for cell counting and 80 µL was used for analysis of Gd (III) content by ICP-MS. The rest of the cell suspension was transferred to flame-sealed glass pipette. The glass pipettes were placed in separate falcon tubes and centrifuged at 500g for 10 min at 4 °C to give the cell pellets at the bottom. The bottom of each pipette was cut out with a glass cutter to give glass capillary tube which was then sealed with parafilm and subjected to MR imaging.

NMRD experiments

Water ^{1}H NMRD profiles were acquired with a Stelar Spinmaster FFC2000-1T relaxometer by measuring the water proton relaxation rates, R_1, as a function of the applied magnetic field (0.01–40 MHz proton Larmor frequency). The R_1 measurements, obtained from the fit of the magnetization decay/recovery curves against a monoexponential function, were affected by an

error below ±1%. The relaxivity profiles were obtained by normalization of the R^1 data, subtracted by the diamagnetic longitudinal relaxation rate contribution, to the Gd^{3+} concentration. The measurements were performed at 25 and 37 °C.

NMRD fitting method

The relaxivity profile of non-aggregated CP1 (r_{1mono}) was extrapolated in the assumption of the presence of only one aggregated form (with relaxivity r_{1aggr}), using the following relationship

$$r_{1obs} = r_{1mono} + (r_{1aggr} - r_{1mono})[Gd^{3+}]/(k + [Gd^{3+}])$$

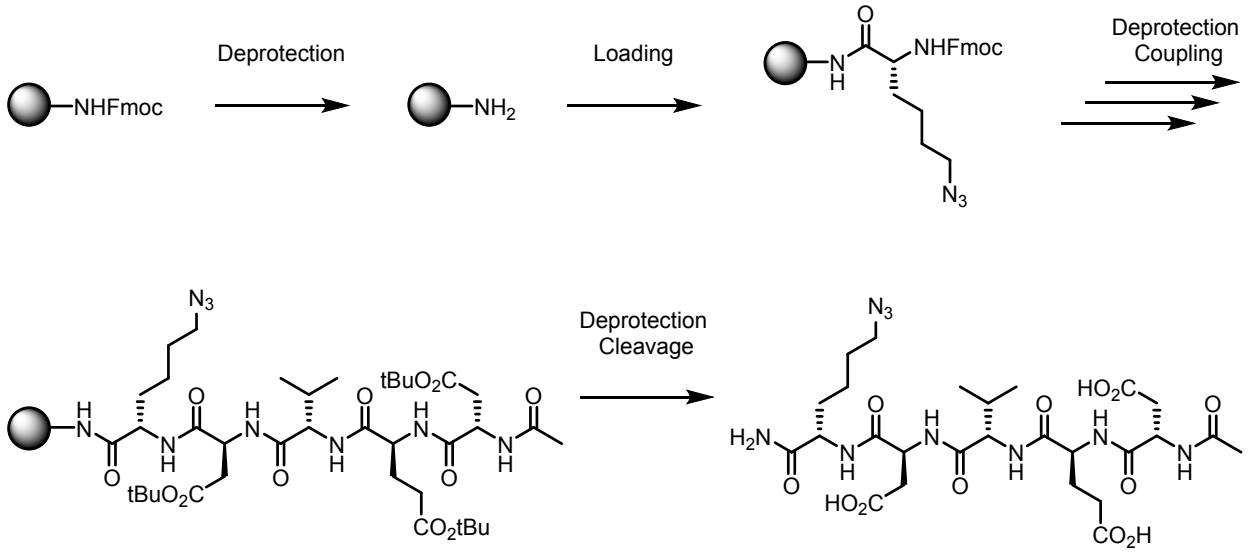
where k, r_{1mono} and r_{1aggr} were fit parameters. Although the accuracy of this simple model may be limited, it provides a very good fit of all collected data.

The relaxivity profiles were fit using the modified Florence NMRD program to account for the presence of static ZFS in slowly reorienting molecules[1,2] and using the Solomon-Bloembergen-Morgan (SBM) model in the case of fast reorienting molecules.[3] Fast internal mobility was introduced with the Lipari-Szabo approach.[4,5]

ICP-MS

Quantification of Gd(III) content in solutions and cell suspensions was accomplished using ICP-MS. Samples were prepared and analyzed according to previously published procedures. [6]

Syntheses



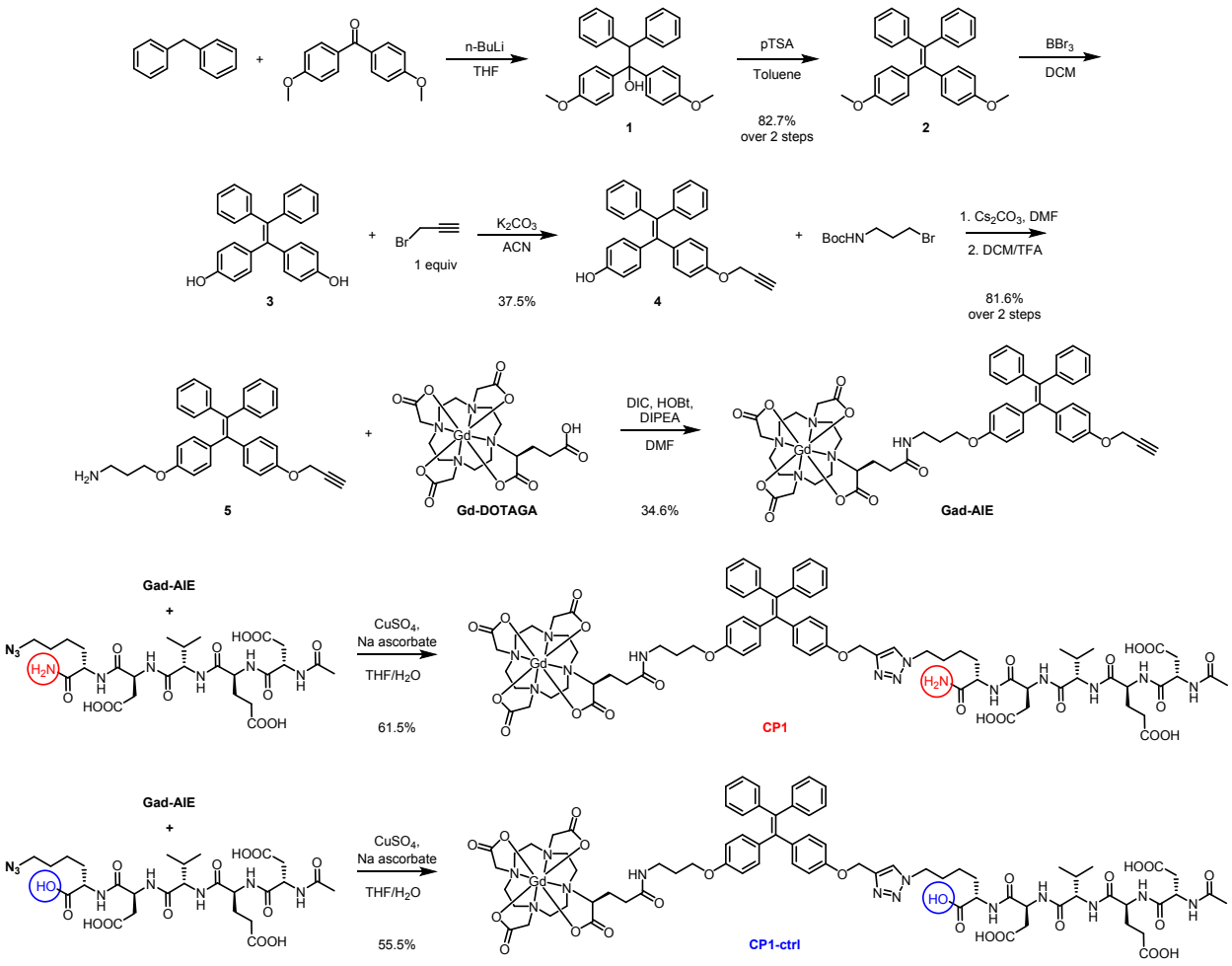
Scheme S1. Synthesis of DEVDK peptide.

a. Deprotection: Rink-amide resin (300 mg, 1.2 mmol/g) was added to a Bio-Rad Poly-Prep chromatography column (20 mL, 0.8×4.0 cm). The resin was suspended in dry DMF (20 mL) and allowed to swell for 1 hr, then dried by passing nitrogen through the vessel. The Fmoc group was removed by shaking the resin in 8 mL of 20% (v/v) piperidine in DMF for 1 hr.

b. Loading the first amino acid resin: the deprotection solution was drained from the resin and a solution of Fmoc-Lys(N_3)-OH (1.0 eq., 82 mg, 0.18 mmol) in 20% 2,4,6-collidine in dry DMF (10 mL) was added and the mixture was gently agitated for overnight. The solution was then drained and a mixture of acetic anhydride:pyridine (3:2, 10 mL) was added immediately. The mixture was gently agitated for 1 h to cap the unreacted sites. The resin was then washed with dry DCM (3 × 10 mL) and dried by passing nitrogen through the vessel.

c. Deprotection and coupling: the Rink amide-Fmoc-Lys(N_3)-OH generated from the previous step was transferred to a peptide synthesis reaction vessel and subjected to cycles of peptide coupling with Fmoc-protected amino acid building blocks. The linear peptide was synthesized from the C-terminus to the N-terminus. Each coupling consisted of i. Fmoc-deprotection with 20% piperidine in DMF for 5 min, ii. washing with DMF (3x), iii. coupling of the amino acid (0.56 mmol, 4 equiv.) in the presence of HBTU (224 mg, 0.56 mmol, 4 equiv.), and iv. washing with DMF (6x). Each amino acid coupling step was 25 min.

d. Peptide cleavage and global deprotection: after the last amino acid was coupled, the resin was washed with DCM (3 × 10 mL) and transferred to a Bio-Rad Poly-Prep chromatography column (20 mL, 0.8×4.0 cm). The linear peptide was cleaved and deprotected by agitating the resin for 1.5 hrs with a solution of TFA/triisopropylsilane (TIPS)/H_2O (18:1:1, 20 mL). The suspension was filtered, washed with additional TFA (10 mL) and the filtrate was collected in a 250 mL round-bottomed flask. The combined filtrates were concentrated by rotary evaporation to give a white film. H_2O (20 mL) was added and white precipitate formed. The suspension was then transferred to a 50 mL centrifuge tube and centrifuged. The supernatant was then filtered through a 0.20 μm syringe filter, freeze-dried and used without further purification.



Scheme S2. Synthesis of CP1 and CP1-ctrl.

1,1-bis(4-methoxyphenyl)-2,2-diphenylethan-1-ol (1). A flame-dried two-neck round-bottom flask was charged with diphenylmethane (6.0 mL, 36.0 mmol, 1.0 eq.) and dry THF (60 mL). A flame-dried addition funnel was attached to the flask and the system was flushed with nitrogen. The reaction was cooled to 0 °C, and 2.5 M *n*-BuLi hexane solution (15.1 mL, 36.0 mmol, 1.05 eq.) was added dropwise via addition funnel over 20 min. A bright yellow/orange solution formed, indicating the formation of carbanion. The reaction was allowed to stir at 0 °C for 2 hrs,

after which bis(4-methoxyphenyl)methanone (8.7 g, 36.0 mmol, 1.0 eq.) was quickly added under nitrogen. The reaction was warmed to room temperature overnight and quenched by slow addition of sat. NH_4Cl (~30 mL) and brine (~50 mL). The aqueous phase was separated and extracted with THF (3 × 50 mL). The combined organic fractions were washed with brine (100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was an off-white solid and was used in the next step without purification.

1,1-bis(4-methoxyphenyl)-2,2-diphenylethan-1-ol (2) Crude **1** was suspended in 150 mL dry toluene with *p*-toluenesulfonic acid monohydrate (1.0 g, 5.3 mmol, 0.15 eq.). The reaction was refluxed for 2 hrs and monitored by TLC. After the reaction was complete, it was cooled to room temperature and directly dry loaded with silica, concentrated, and purified via flash chromatography on a silica column (gradient, 2:15:83 to 5:20:75 to 10:20:70 v/v/v EtOAc:DCM:Hexanes) to afford the title compound as a light yellow solid (11.7 g, 29.8 mmol, 82.7% yield over two steps). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.24 (m, 6H), 7.20 – 7.14 (m, 4H), 7.09 (d, J = 8.7 Hz, 4H), 6.79 (d, J = 8.7 Hz, 4H), 3.89 (s, 6H). ^{13}C NMR (125 MHz, 500 MHz, $CDCl_3$) δ 158.04, 144.29, 140.08, 139.23, 136.36, 132.57, 131.37, 127.66, 126.05, 112.99, 55.09. HR-MS calculated for $C_{28}H_{25}O_2$ $[M+H]^+$ *m/z* 393.1855, found 393.1841.

4,4'-(2,2-diphenylethene-1,1-diyl)bis(methoxybenzene) (3) A flame-dried round-bottom flask charged with **2** (5.0 g, 12.7 mmol, 1.0 eq.) and dry DCM (60 mL) was cooled to 0 °C . A flame-dried addition funnel was attached to the flask and charged with 20 mL dry DCM. Under nitrogen, neat BBr_3 (3.2 mL, 33.1 mmol, 2.6 eq.) was injected into the addition funnel. BBr_3 in DCM solution was then added to the reaction dropwise over 1 hr. After overnight stirring at

room temperature, the reaction was quenched by slow addition of ice cold sat. $NaHCO_3$ (~100 mL). The DCM was evaporated off and the aqueous phase was extracted with EtOAc (3 × 100 mL). The combined organic fractions were washed with brine (100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was a light pink solid (99 % crude yield) with > 95% purity as determined by NMR and was used in the next step without purification. ^{1}H NMR (500 MHz, $CD_3OD/CDCl_3$) δ 7.04 (m, 6H), 6.99 (m, 4H), 6.83 (m, *J* = 8.6 Hz, 4H), 6.52 (d, *J* = 8.6 Hz, 4H). ^{13}C NMR (125 MHz, $CD_3OD/CDCl_3$) δ 155.14, 144.49, 140.61, 138.45, 135.46, 132.59, 131.28, 127.46, 125.74, 114.29. HR-MS calculated for $C_{26}H_{21}O_2$ $[M+H]^+$ *m/z* 365.1542, found 365.1526.

4-(2,2-diphenyl-1-(4-(prop-2-yn-1-yloxy)phenyl)vinyl)phenol (4) A round-bottom flask charged with compound **3** (5.0 g, 13.7 mmol, 1.0 eq.), K_2CO_3 (5.7 g, 41.1 mmol, 3.0 eq.) and dry ACN (50 mL) was heated at 75 °C. A flame-dried addition funnel was attached to the flask and charged with 20 mL dry ACN. Propargyl bromide solution 80 wt. % in toluene (1.8 mL, 16.2 mmol, 1.2 eq.) was injected into the addition funnel. The Propargyl bromide solution was then added to the reaction dropwise over 30 min. The reaction was refluxed for another 4 hrs, cooled to room temperature and filtered through a bed of celite. The celite was washed with additional ACN (3 × 50mL). The filtrate was dried (Na_2SO_4), filtered, dried loaded with silica and concentrated under reduced pressure. The crude product was purified via flash chromatography on a silica column (gradient, 10:15:75 to 15:15:70 v/v/v EtOAc:DCM:Hexanes) to afford the title compound as a yellow fluffy powder (2.0 g, 5.0 mmol, 37.3 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 – 7.20 (m, 6H), 7.17 (d, *J* = 2.3 Hz, 4H), 7.11 (d, *J* = 8.6 Hz, 2H), 7.05 (d, *J* = 8.5 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 6.71 (d, *J* = 8.5 Hz, 2H), 4.82 (s, 1H), 4.76 (d, *J* = 2.4 Hz, 2H),

2.64 (d, J = 2.4 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.13, 153.99, 144.15, 144.07, 139.80, 139.61, 137.16, 136.49, 132.78, 132.55, 131.33, 127.70, 127.68, 126.15, 114.57, 113.95, 78.58, 75.44, 55.78. HR-MS calculated for $C_{29}H_{21}O_2$ $[M-H]^-$ *m/z* 401.1547, found 401.1534.

3-(4-(2,2-diphenyl-1-(4-(prop-2-yn-1-yloxy)phenyl)vinyl)phenoxy)propan-1-amine (5) A round-bottom flask charged with compound **4** (1.0 g, 2.5 mmol, 1.0 eq.), 3-(Boc-amino)propyl bromide (769 mg, 3.2 mmol, 1.3 eq.), Cs_2CO_3 (1.6 g, 5.0 mmol, 2.0 eq.) and dry DMF (10 mL) was stirred at r.t for 6 hrs. The reaction mixture was concentrated, re-dissolved in DCM and filtered through a bed of celite. The celite was washed with additional DCM (3 × 20mL). The filtrate was dried (Na_2SO_4), filtered, concentrated and dissolved in 30 mL TFA/DCM (2:1 v/v) for Boc deprotection. The reaction mixture was stirred at r.t. for 5 hrs, concentrated and was slowly added sat. sodium bicarbonate (20 mL). The aqueous phase was extracted with DCM (3 × 30 mL). The combined organic fractions were washed with brine (50 mL), dried (Na_2SO_4), filtered, concentrated and purified via flash chromatography on a silica column (gradient, 7:93 to 10:90 to 15:85 to 20:80 v/v MeOH: DCM) to afford the title compound as a dark yellow solid (930 mg, 2.0 mmol, 81.6 % yield over two steps). It is worth noticing that when ACN was used as the solvent for the first alkylation step, much lower yield was obtained. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.10 – 7.02 (m, 6H), 7.00 – 6.94 (m, 4H), 6.93 – 6.86 (m, 4H), 6.65 (d, J = 8.8 Hz, 2H), 6.62 (d, J = 8.6 Hz, 2H), 4.56 (d, J = 2.4 Hz, 2H), 3.93 (t, J = 5.7 Hz, 2H), 3.17 (t, J = 7.0 Hz, 2H), 2.46 (t, J = 2.4 Hz, 1H), 2.16 (p, J = 6.5 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.80, 156.12, 144.06, 139.73, 139.69, 137.14, 136.83, 132.61, 132.53, 131.32, 127.73, 127.69, 126.19, 126.16, 113.96, 113.63, 75.45, 65.14, 55.75, 38.06, 27.37. HR-MS calculated for $C_{32}H_{30}NO_2$ $[M+H]^+$ *m/z* 460.2277, found 460.2262.

Gad-AIE A round-bottom flask charged with **Gd-DOTAGA** (246.6 mg, 0.392 mmol, 1.2 eq.), *N,N'*-diisopropylcarbodiimide (81 uL, 0.522 mmol, 1.6 eq.), hydroxybenzotriazole (HOBt) (88.1 mg, 0.652 mmol, 2 eq.), DIPEA (189 uL, 0.978 mmol, 3.0 eq.) and dry DMF (6 mL) was stirred at r.t. until a homogeneous solution formed and **5** (150.0 mg, 0.326 mmol, 1.0 eq.) was then added. The resulting mixture was stirred overnight, concentrated, dried, re-dissolved in 10 mL ACN/H_2O (v/v =1:9) and purified by reverse phase HPLC (XBridge C18 column) held at 10% for 5 min and eluting with a gradient of 10%−55% acetonitrile (0.1% NH_4OH) in water (0.1% NH_4OH) over 30 min, t_r = 27.8 min. This gave about 200 mg of the product as a white solid after lyophilization. The purity and identity of the product was confirmed using analytical HPLC-MS on a C18 column, reverse phase HPLC (XBridge C18 column) held at 0% for 3 min and eluting with a gradient of 0%−100% acetonitrile (0.1% NH_4OH) in water (0.1% NH_4OH) over 18 min, t_r = 14.7 min. HR-MS calculated for $C_{51}H_{55}GdN_5O_{11}$ $[M]^-$ *m/z* 1071.3139, found 1071.3173.

CP1 A round-bottom flask charged with **Gad-AIE** (110.0 mg, 0.103 mmol, 1.0 eq.), DEVD peptide (82.8 mg, 0.123 mmol, 1.2 eq.), $CuSO_{4\cdot5}H_2O$ (25.8 mg, 0.103 mmol, 1.0 eq.), sodium ascorbate (24.5 mg, 0.123 mmol, 1.2 eq.) and 12 mL THF/H2O (v/v = 1:1) was stirred at r.t. overnight. The reaction mixture was concentrated, dried, re-dissolved in 10 mL H_2O (0.1% NH_4OH) and purified by reverse phase HPLC (XBridge C18 column) held at 0% for 5 min and eluting with a gradient of 0%−10% acetonitrile (0.1% NH_4OH) in water (0.1% NH_4OH) over 5 min, then 10%−30% over 30 mins, t_r = 18.5 min. This gave 123 mg of the product as a white solid after lyophilization. The purity and identity of the product was confirmed using analytical HPLC-MS on a C18 column, reverse phase HPLC (XBridge C18 column) held at 0% for 3 min and eluting with a gradient of 0%−100% acetonitrile (0.1% NH_4OH) in water (0.1% NH_4OH)

over 18 min, t_r = 10.0 min. HR-MS calculated for $C_{77}H_{96}GdN_{14}O_{23}$ $[M]^-$ *m/z* 1742.6014, found 1742.5985.

Table S2. Fluorescence properties of compound CP1, Gad-AIE and quinoline sulfate

Compound	**Φ**	**λ_{abs} (nm)**	**λ_{em} (nm)**
Quinoline sulfate	0.546	346	452
Gad-AIE	0.023	313	476
CP1	<0.003	308	476

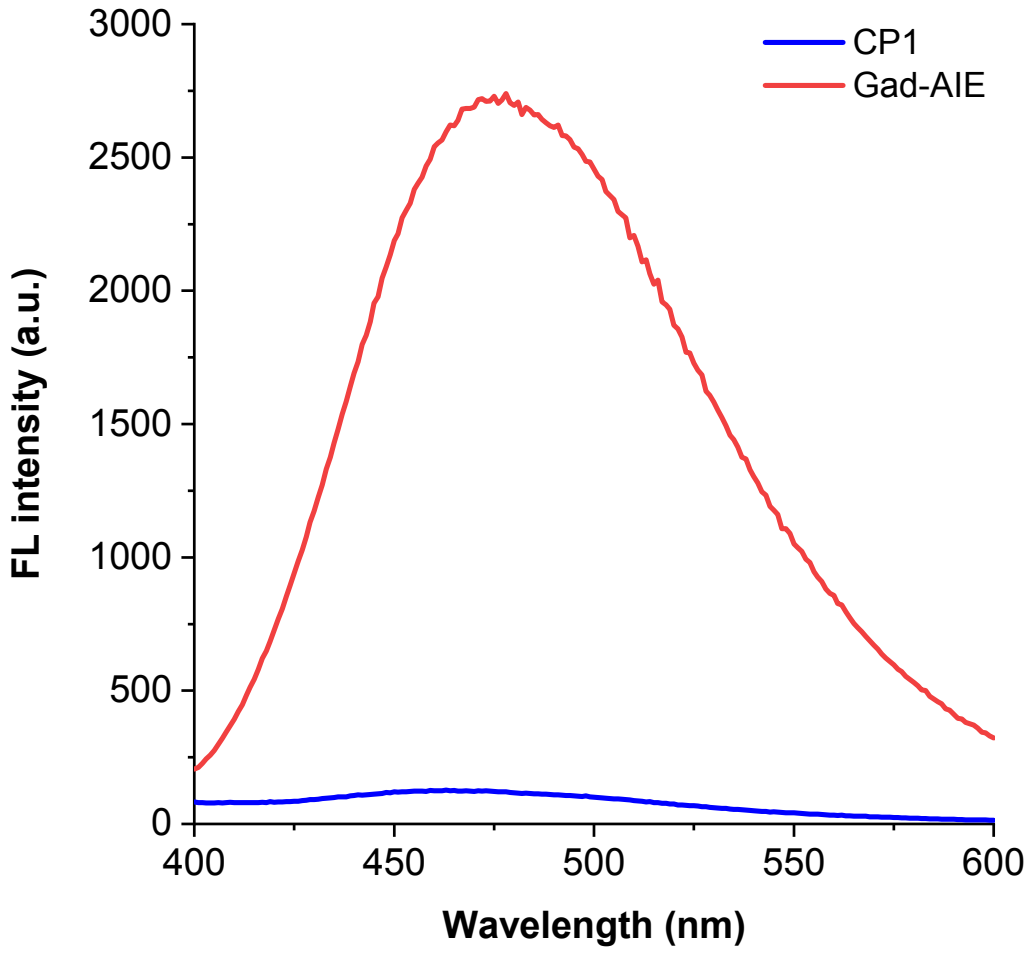


Figure S1. FL spectra of CP1 and Gad-AIE at 200 μM.

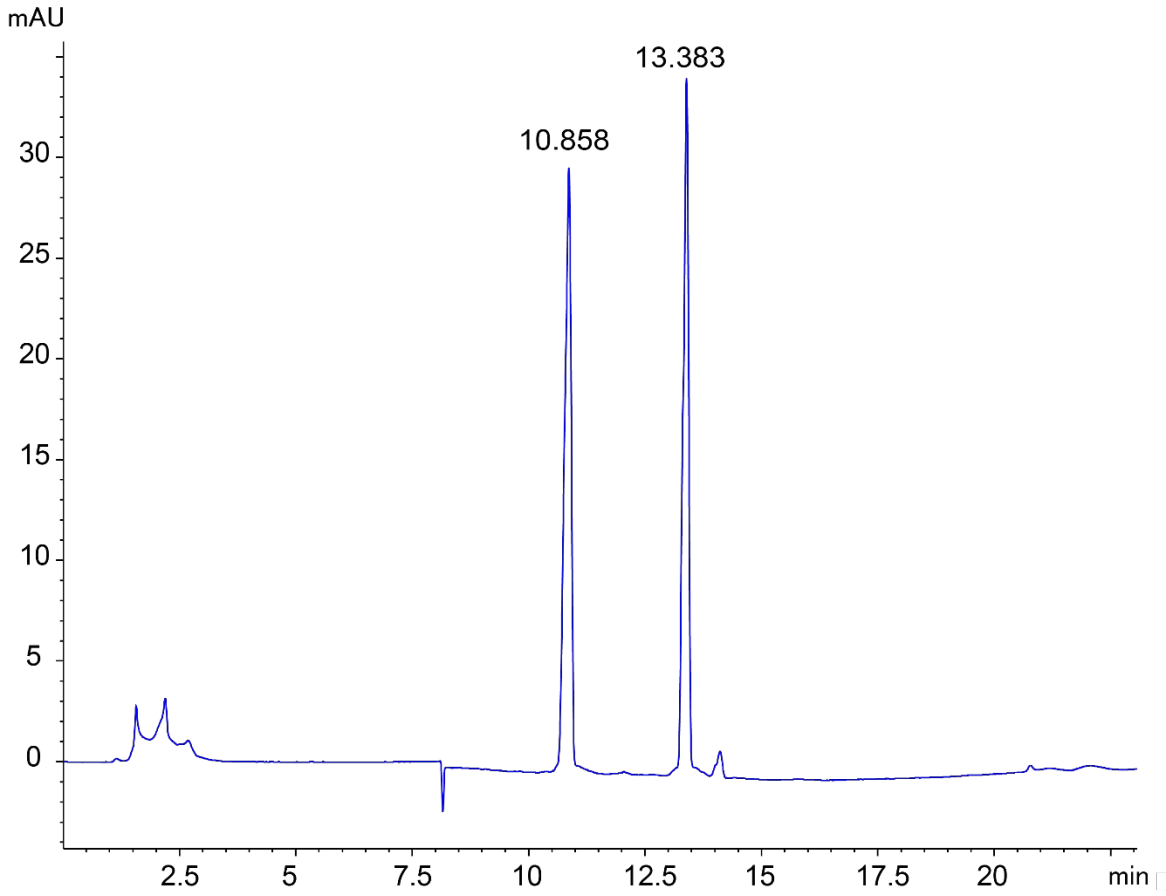


Figure S2. Analytical trace for CP1 incubated with caspase-3 overnight.

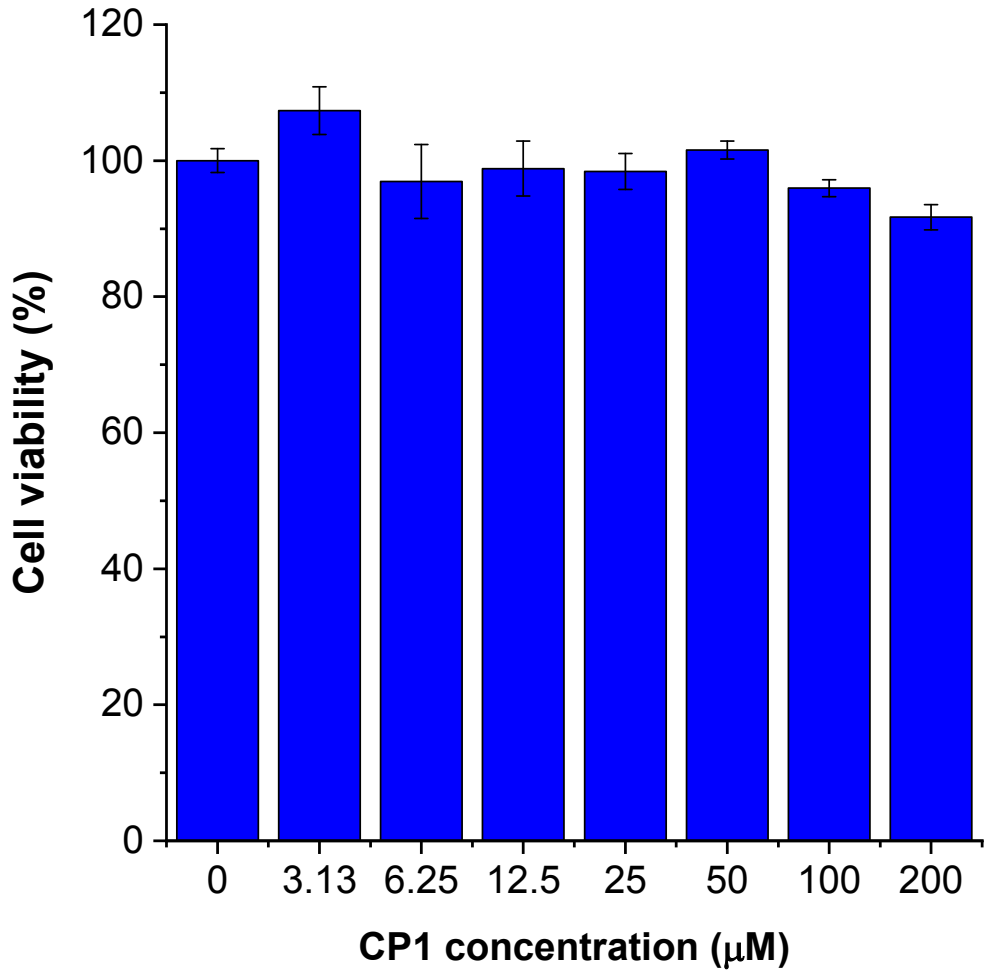


Figure S3. Toxicity of CP1 was determined with MTS assay. HeLa cells were incubated with CP1 at concentrations ranging from 0 to 200 μM for 24 h and showed ≥ 90% viability at the highest CP1 concentration (200 μM).

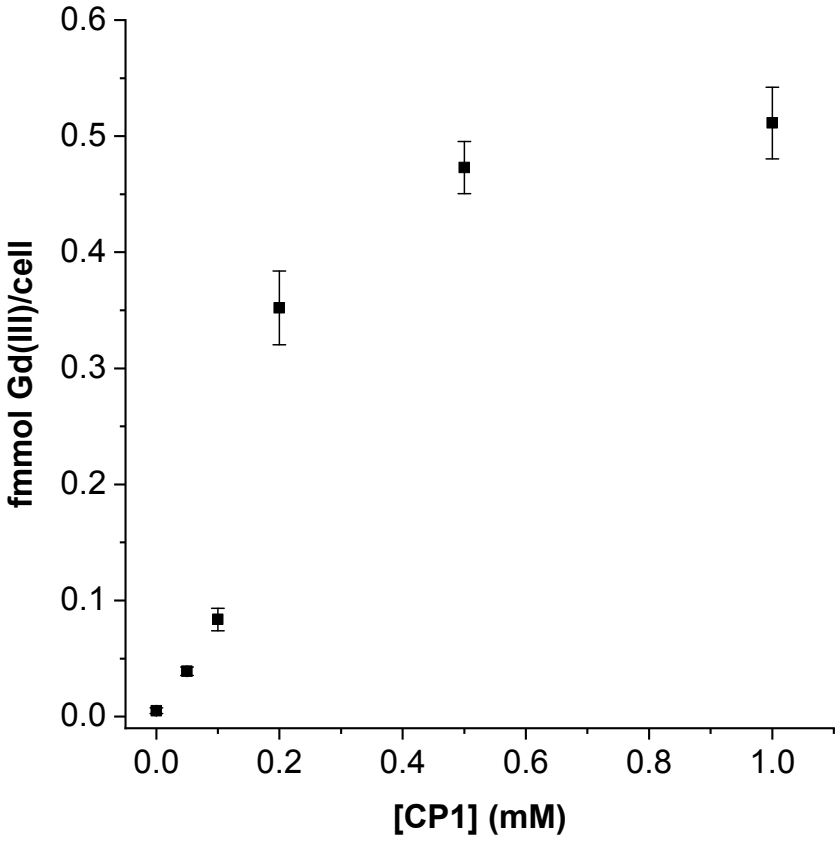


Figure S4. Concentration-dependent uptake of CP1 (24 hrs incubation) in HeLa cells. These data show that maximum uptake of CP1 is reached at about 0.5 mM.

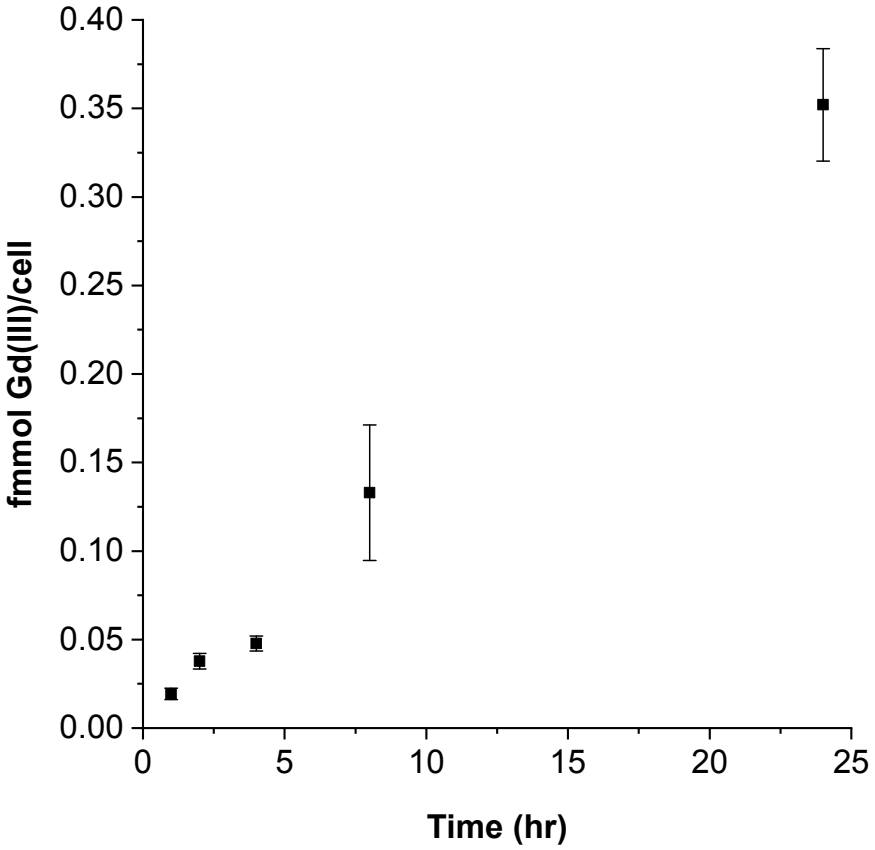


Figure S5. Time-dependent uptake of CP1 (0.2 mM) in HeLa cells. These data reveal that uptake of CP1 in HeLa cells is time-dependent.

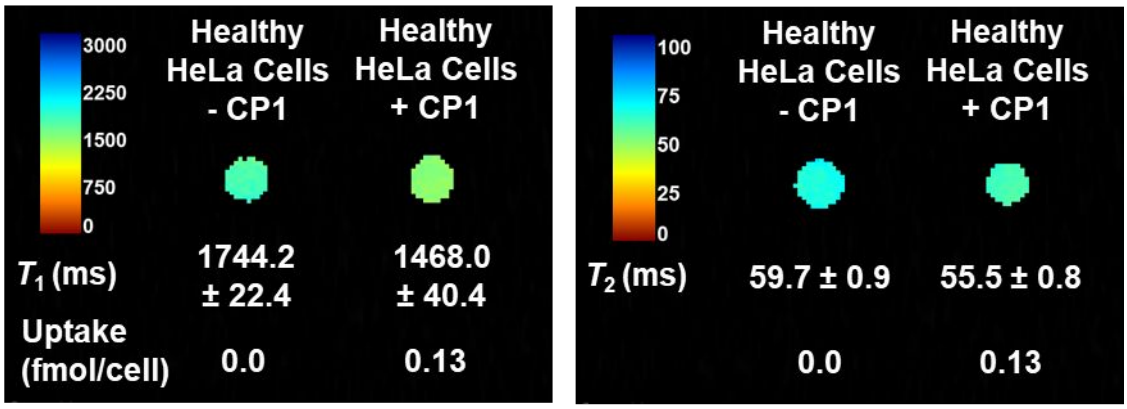


Figure S6. MR imaging (7 T) of healthy HeLa cell pellets incubated with and without CP1. This data suggests that enough CP1 accumulated in cell to produce both T_1 and T_2 MR signals.

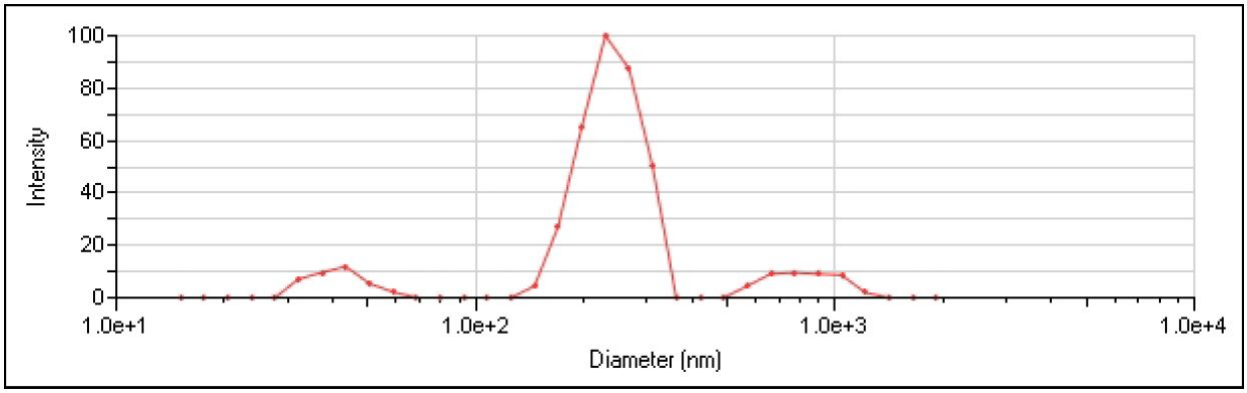


Figure S7. DLS measurement of 5 mM CP1 in water. The mean diameter of CP1 particles from 6 measurements is 199.8 ± 4.9 nm.

Table S3. Best fit of the relaxivity profiles reported in Fig. 6C.

	Gad-AIE, 25 °C	Gad-AIE, 37 °C	CP1 (extrap.), 25 °C	CP1 (extrap.), 37 °C
Δ_t (cm^{-1})	0.0089	0.0089	0.0089 (*)	0.0089 (*)
τ_R (ps)	3500	2700	100	71
S^2	0.49	0.49		
τ_f (ps)	980	350		
τ_v (ps)	29	26	29 (*)	26 (*)
τ_M (ns)	**350**	**300**	**350**	**300**
***r* (Å)**	**3.1**	**3.1**	2.83(**)	2.83(**)
ZFS (cm^{-1})	0.029	0.029		
θ	20°	20°		
***d* (Å)**			**3.6**	**3.6**
***D* (10^{-9} m^2/s)**			**2.5**	**3.5**

Values in bold were fixed in the fit

(*) fixed to the value obtained from the fit of Gad-AIE

(**) This short *r* may be due to some inaccuracy in the extrapolation, and/or to the presence of second sphere contributions. For instance, the contribution from two additional water molecules in very fast exchange (<100 ps) at 3.6 Å provides an equally good fit with r = 3.1 Å.

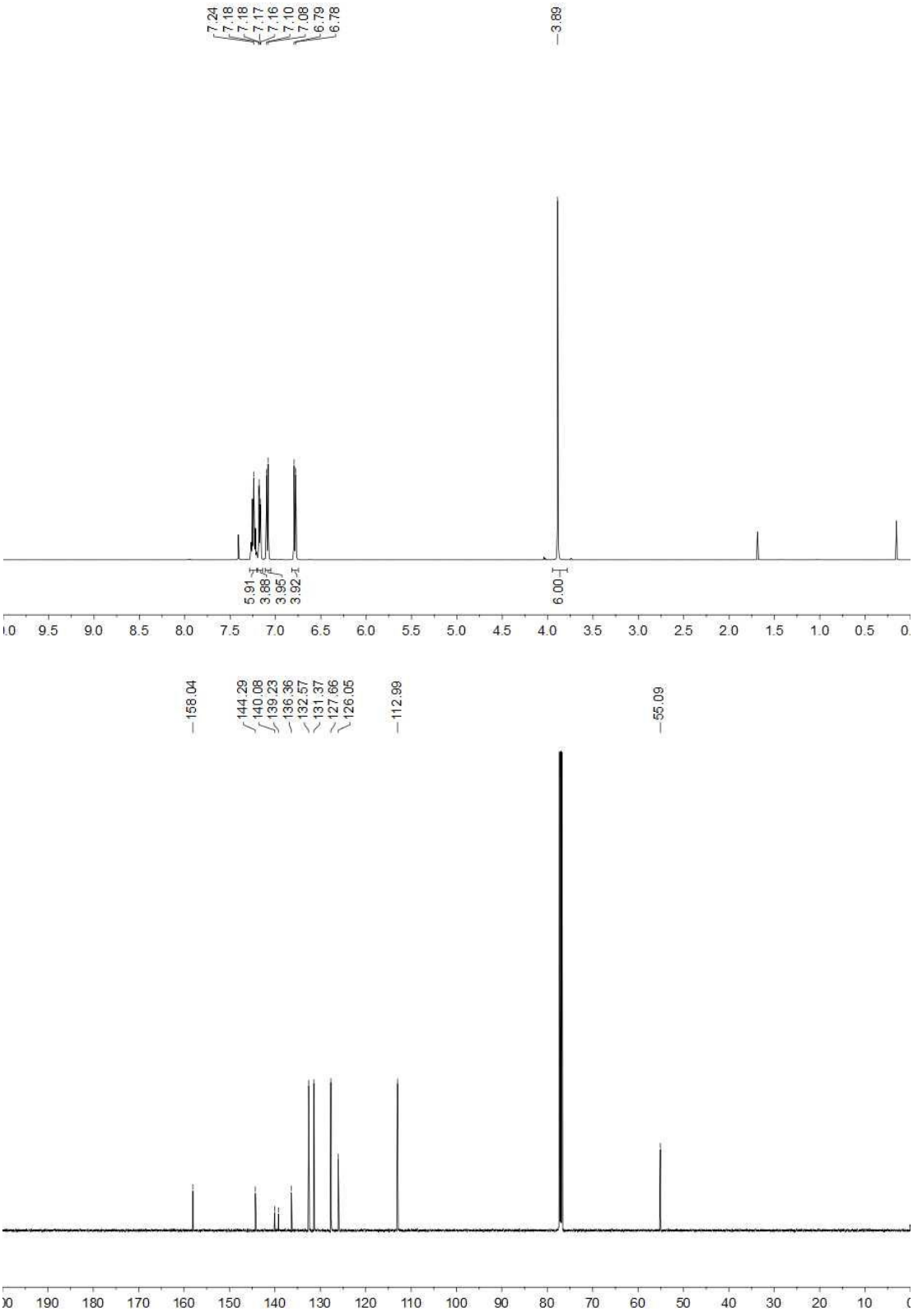


Figure S8. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **2**.

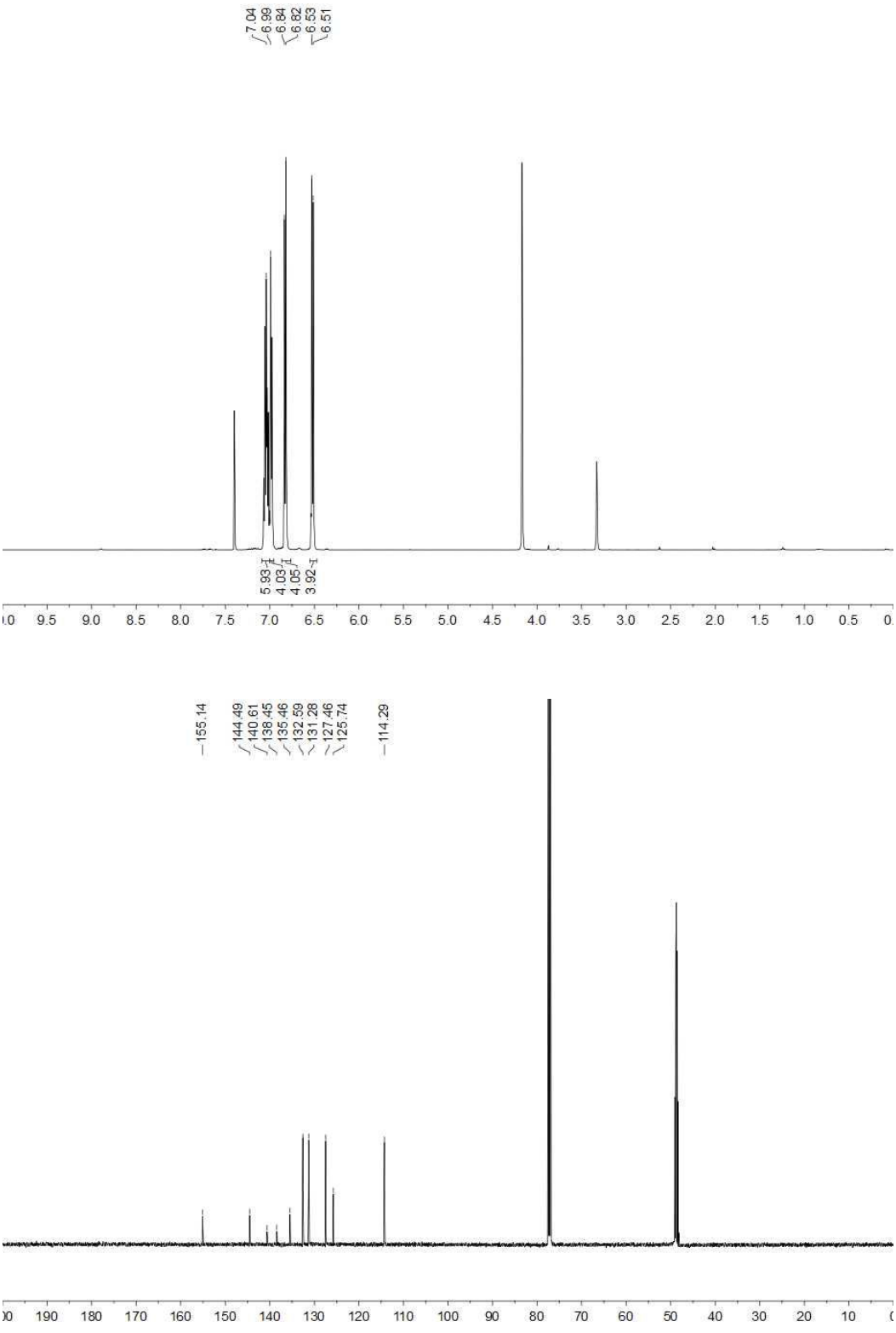


Figure S9. ^{1}H NMR (500 MHz, $CD_3OD/CDCl_3$) and ^{13}C NMR (125 MHz, $CD_3OD/CDCl_3$) NMR spectra of **3**.

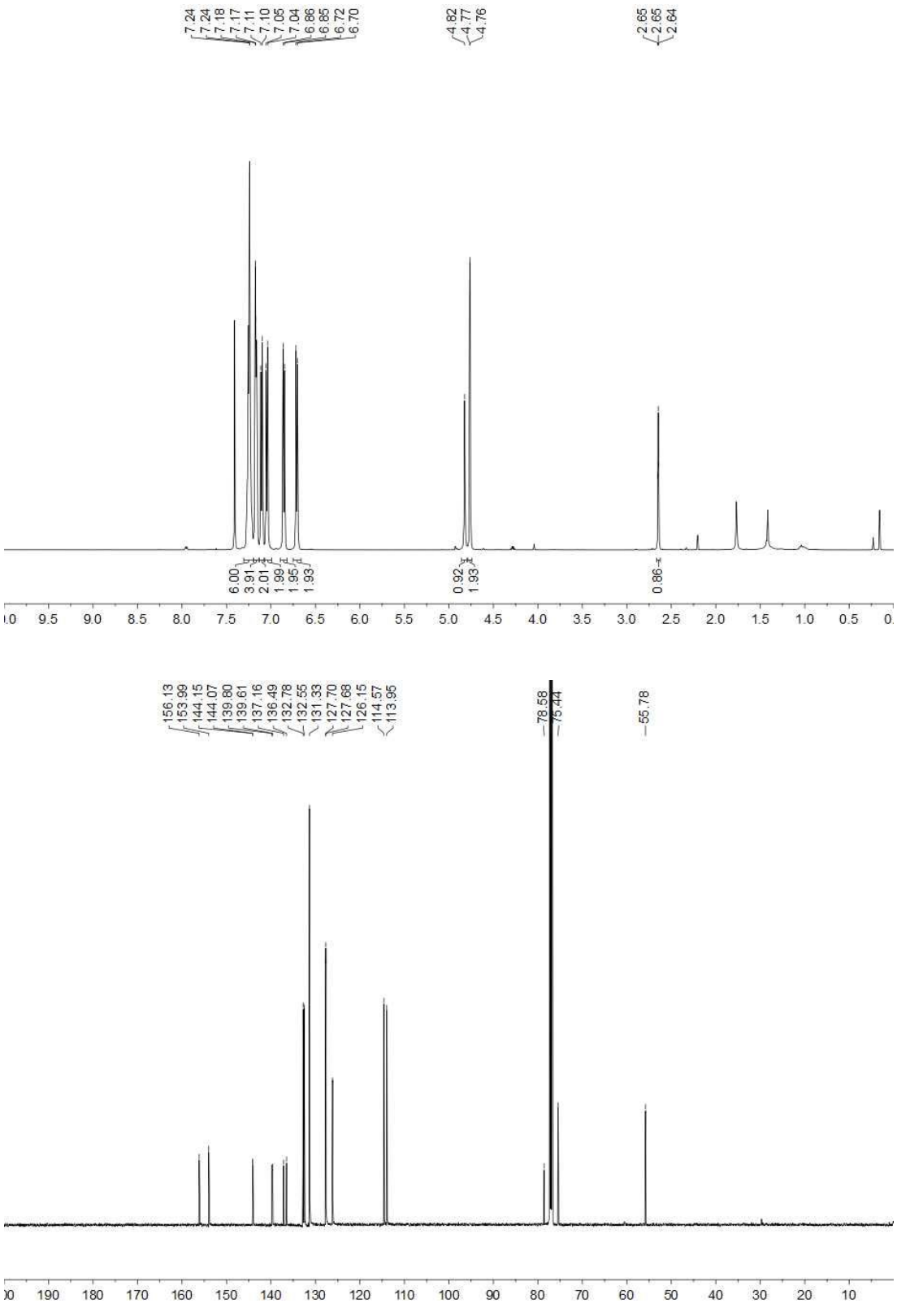


Figure S10. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **4**.

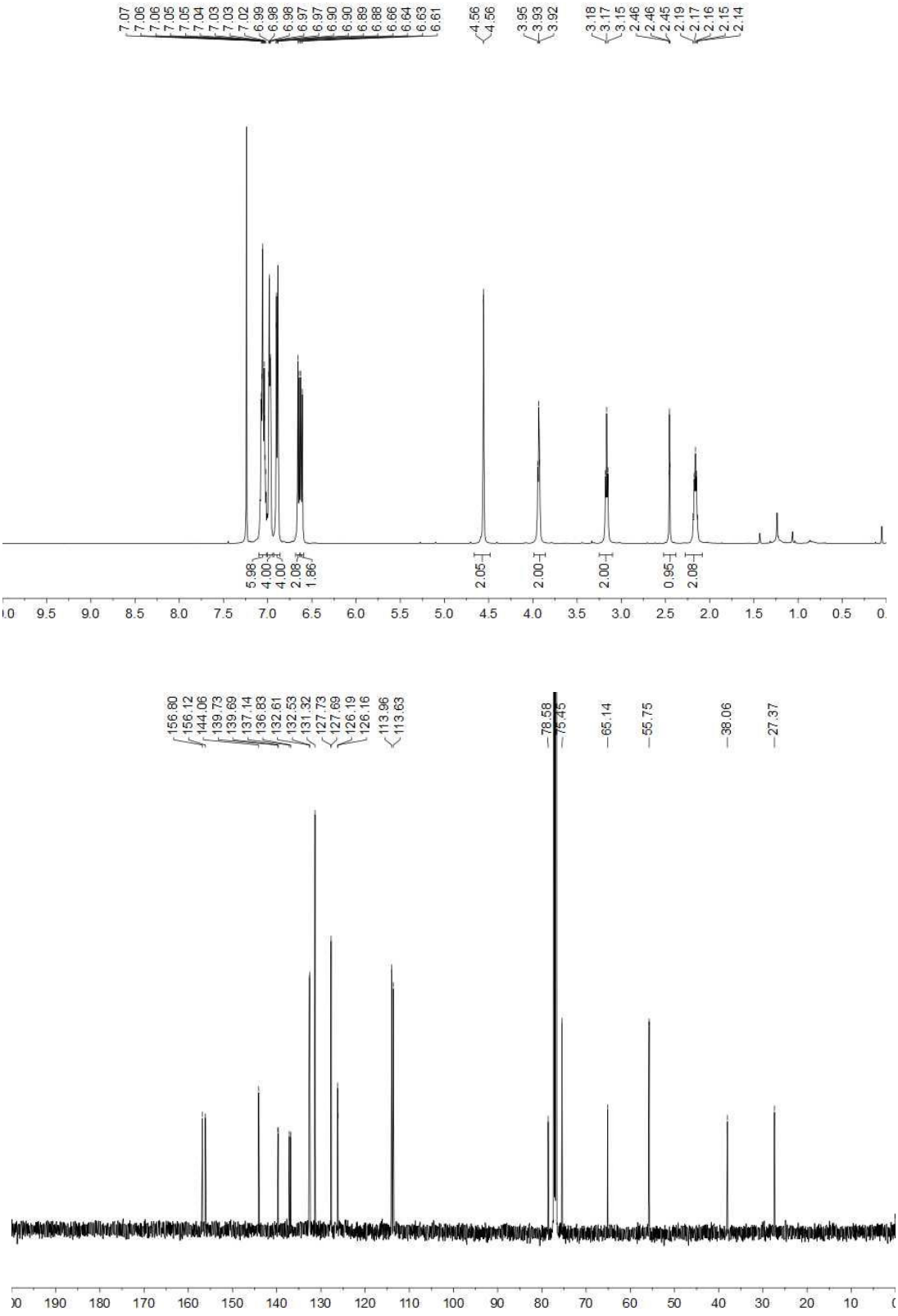


Figure S11. ^{1}H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$) NMR spectra of **5**.

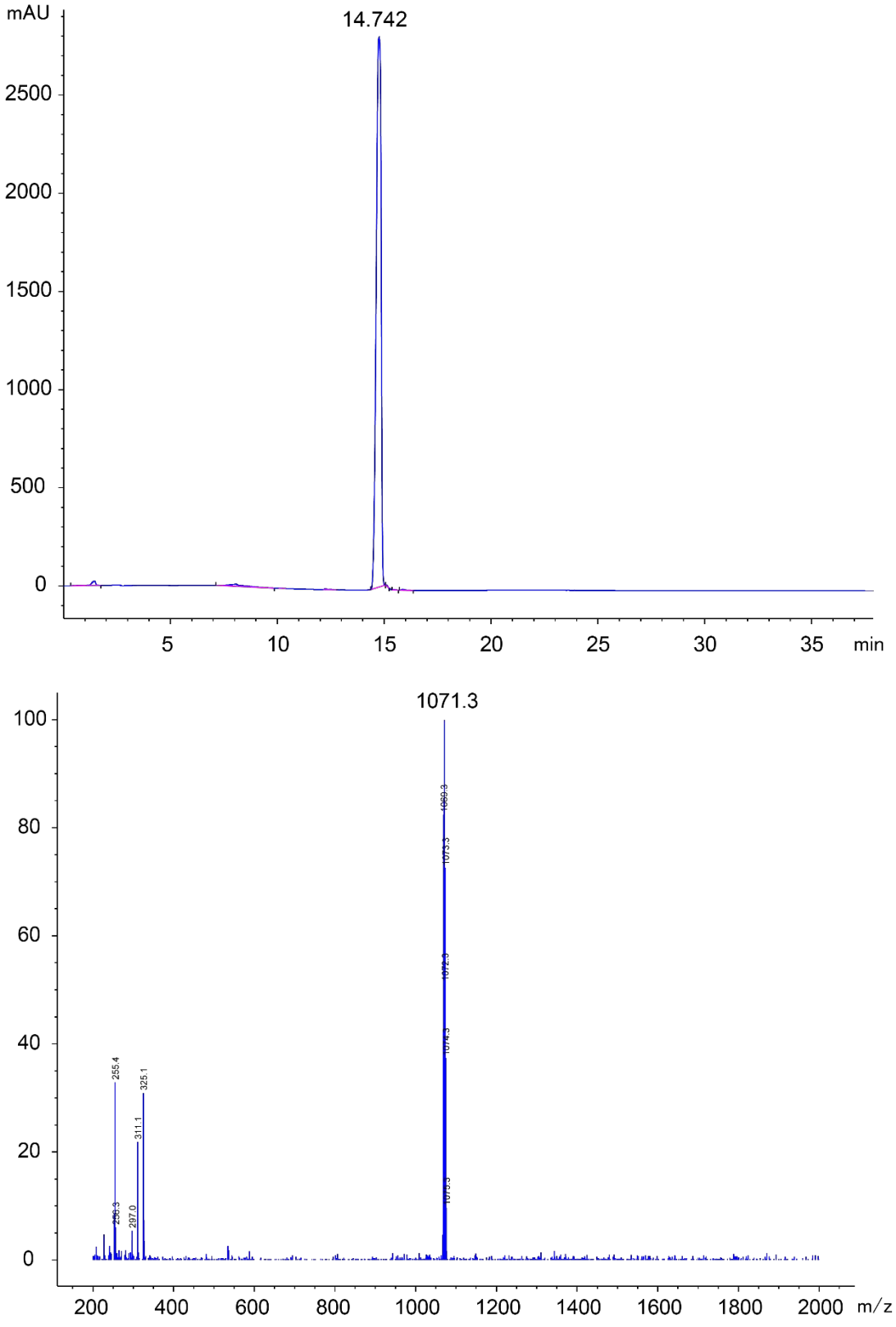


Figure S12. Analytical trace of Gad-AIE and its corresponding ESI-MS spectra.

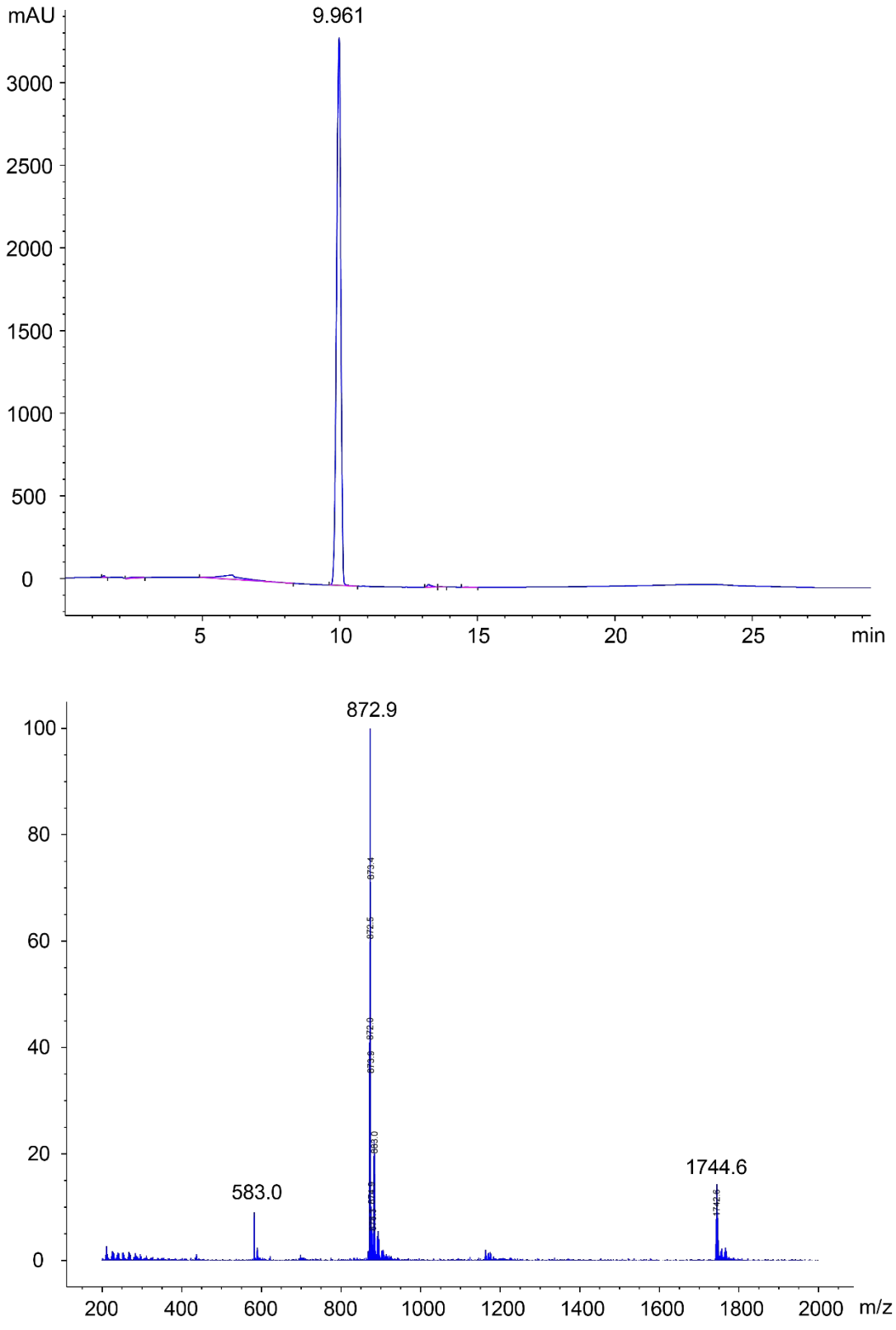


Figure S13. Analytical trace of CP1 and its corresponding ESI-MS spectra.

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