

Supporting Information

Caspase-responsive smart gadolinium-based contrast agent for magnetic resonance imaging of drug-induced apoptosis[†]

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Fig. S1 Characterization of caspase-3-cleavable but non-condensable control probe **1-ctrl** *in vitro*. (a) Proposed molecular transformation of **1-ctrl** upon incubation with caspase-3. (b) HPLC traces of **1-ctrl** (black) and treatment of **1-ctrl** (200 μ M) with recombinant human caspase-3 (50 nM) for 24 h at 37 $^{\circ}$ C in enzyme reaction buffer (pH 7.4) (red). (c) MS spectra (ESI) of HPLC of the compounds corresponding to the HPLC peaks (peaks * and **) in (b). HPLC and MS analysis show that **1-ctrl** was converted to **1-ctrl-r** after DEVD peptide cleavage and disulfide reduction.

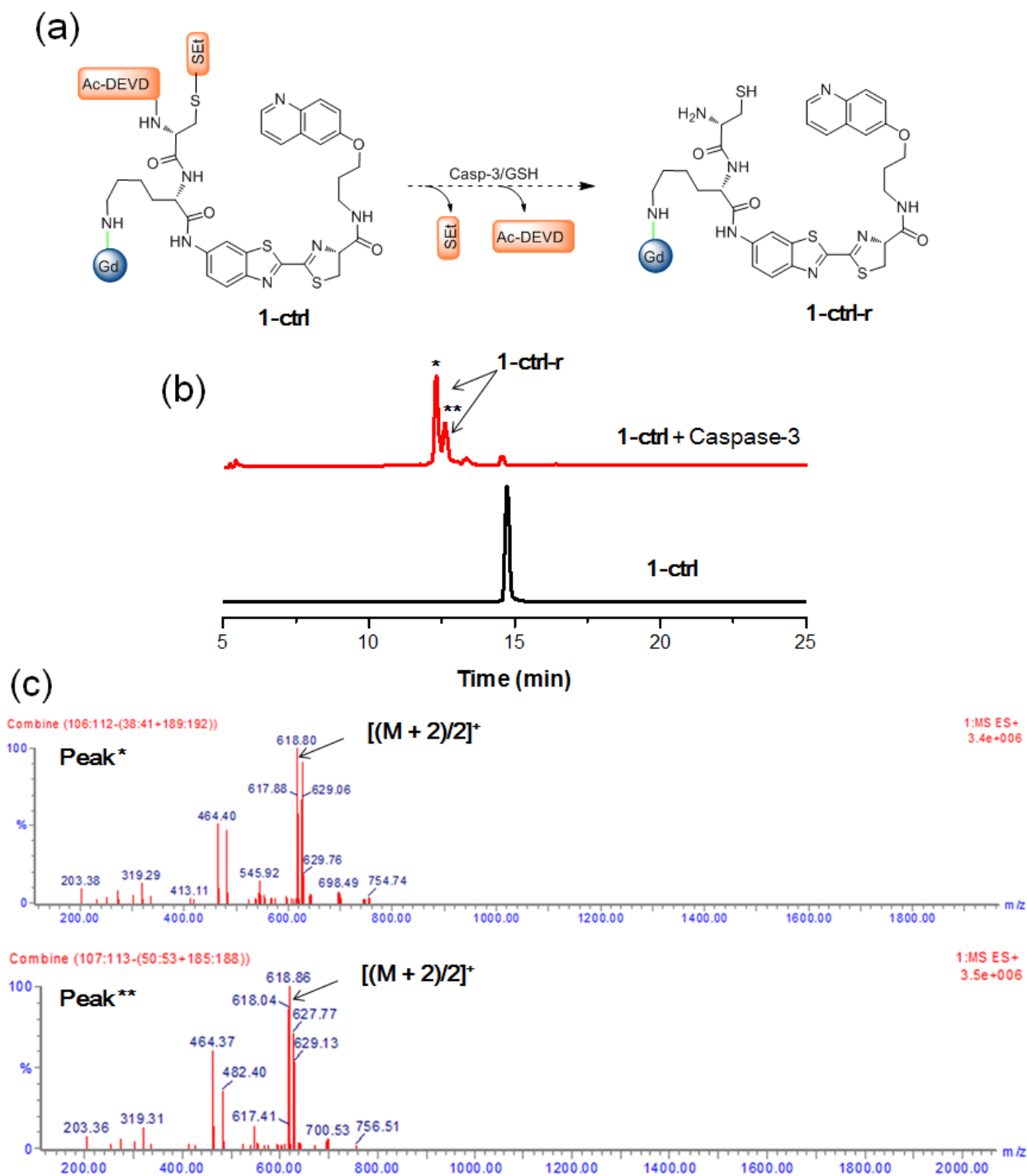


Fig. S2 Energy-dispersive X-ray (EDX) spectroscopy analysis of the nanoparticles in Figure 2c shows the presence of Gd element signal from the particles.

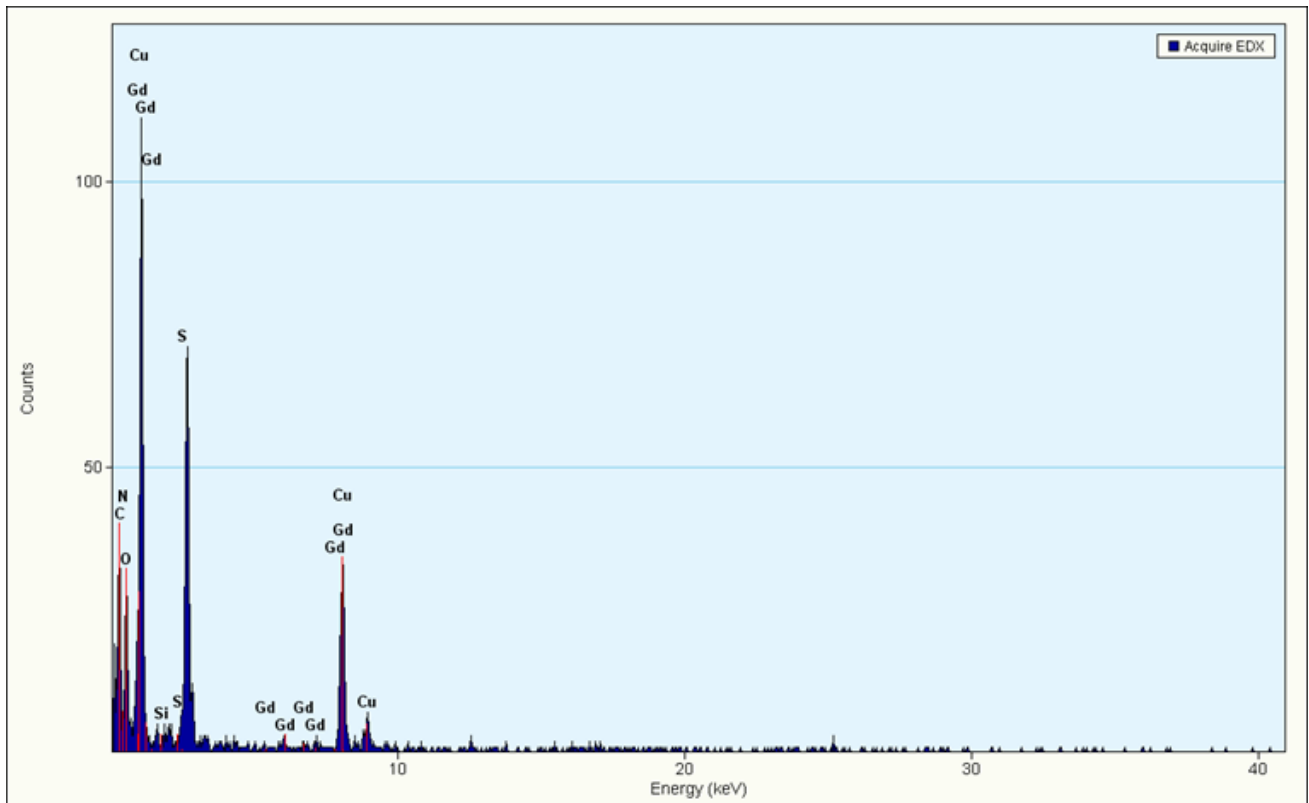


Fig. S3 Plots of R_1 vs Gd concentration to determine the relaxivity of **1** and **1-ctrl** at 1 T (a), 1.5 T (b) and 3 T (c). Varying concentrations of **1** and **1-ctrl** (50 – 500 μM) were incubated with or without caspase-3 (50 nM) in enzyme reaction buffer at 37 $^\circ\text{C}$ overnight. The resulting solutions were scanned using the standard inversion recovery spin-echo sequence on 1 T, 1.5 T and 3 T MRI scanners. Signal intensity versus TI relationships were fit to the following exponential T1 recovery model by non-linear least squares regression: $SI(TI) = S_0 [1 - 2 \cdot \exp(-TI/T_1) + \exp(-TR/T_1)]$. Relaxation rates (R_1) were determined as $1/T_1$. The true concentrations of Gd in the solutions were further confirmed by ICP-MS measurement. The longitudinal relaxivities (r_1 , units of $\text{mM}^{-1}\text{s}^{-1}$) were determined as the slope of R_1 vs. incubation Gd concentration of each sample. The relaxivity values are summarized in Table 1.

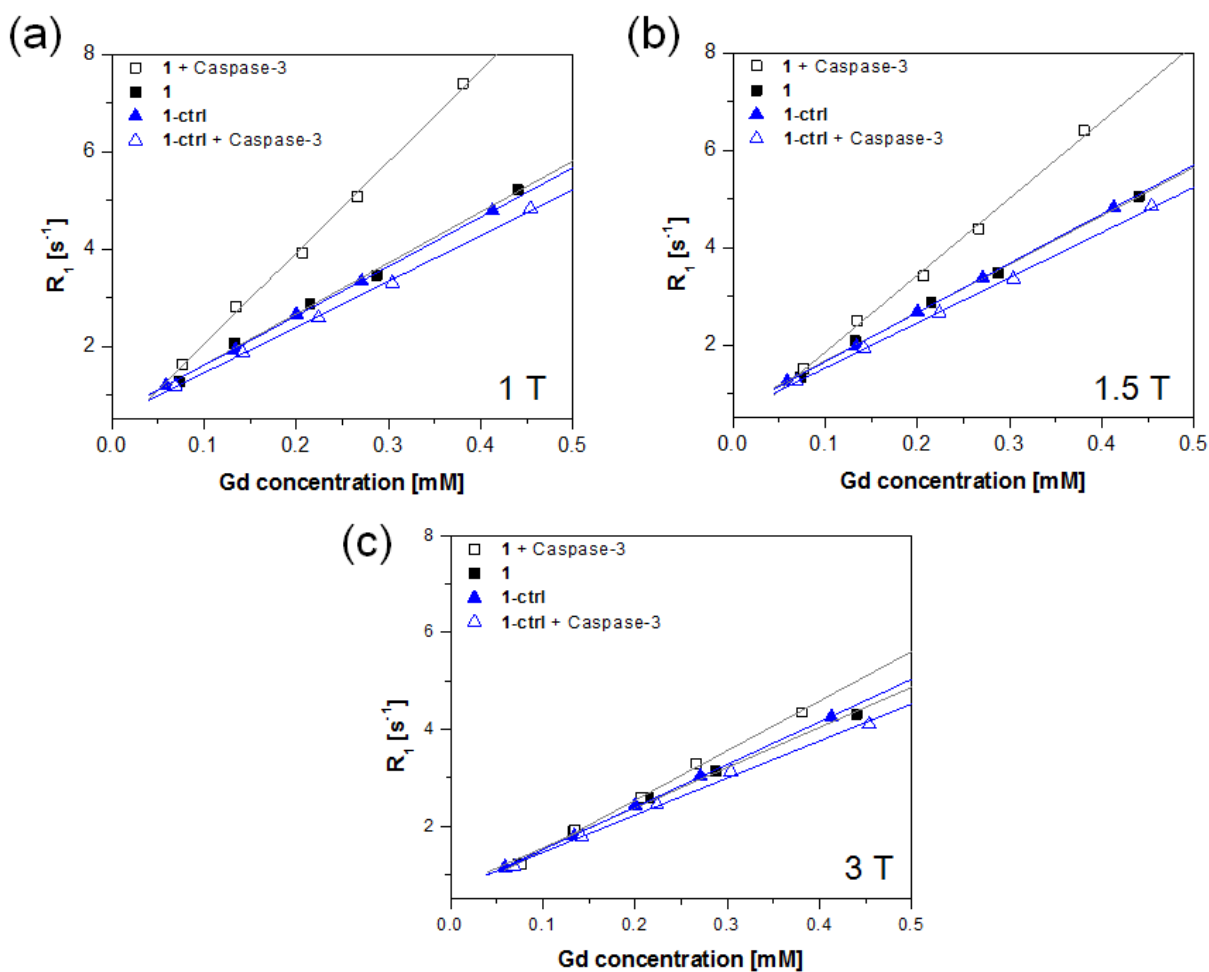


Fig. S4 The T_1 values (1 T) of the incubation solution of **1** (208 μM) with different relevant proteases (50 nM) and caspase-3 together with inhibitor Z-VAD-fmk in enzyme reaction buffer (pH 7.4) for 24 h. T_1 values were measured at 1T scanner (Bruker Icon, Bruker BioSpin Corp.) at r.t., using a series of inversion-prepared fast spin-echo scans. There was a significant reduction in T_1 in the solution after incubation with caspase-3 and -7, which correlates well with the brighter T_1 -weighted images.

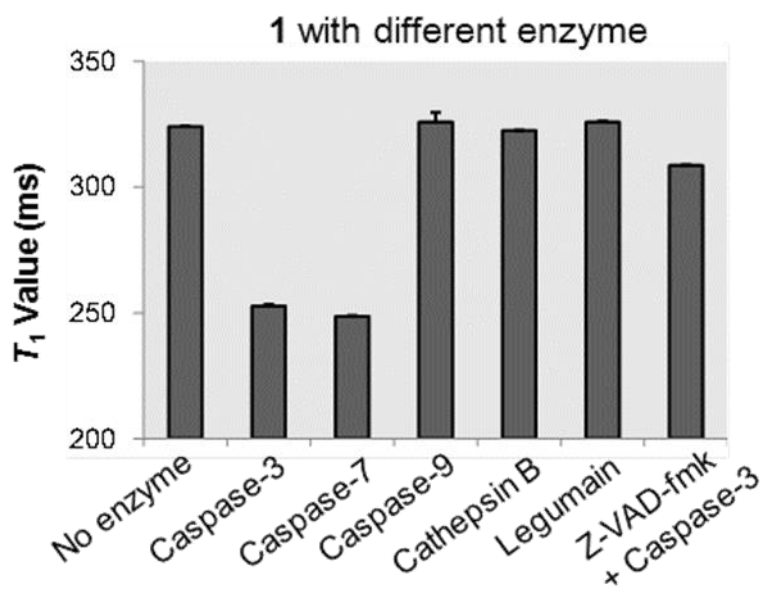


Fig. S5 Enzymatic specificity studies of C-SNAM *in vitro*. (a) Brighter T_1 -weighted images of C-SNAM were produced by caspase-3 than other selected proteases (Caspase-1, Cathepsin D, L and S). C-SNAM (192 μM) was incubated with indicated protease (50 nM) in enzyme reaction buffer (pH 7.4) at 37 $^\circ\text{C}$ overnight. T_1 -weighted spin-echo images (TE/TR = 6/6000 ms) of the incubation solutions were acquired at 1 T at r.t. (b) The T_1 values (1 T) of the incubation solution of C-SNAM (192 μM) with different relevant proteases (50 nM Caspase-3, -1, Cathepsin D, L and S) in enzyme reaction buffer (pH 7.4) for 24 h. T_1 values were measured at 1T scanner (Bruker Icon, Bruker BioSpin Corp.) at r.t., using a series of inversion-prepared fast spin-echo scans. There was a significant reduction in T_1 in the solution after incubation with caspase-3, which correlates well with the brighter T_1 -weighted images in (a).

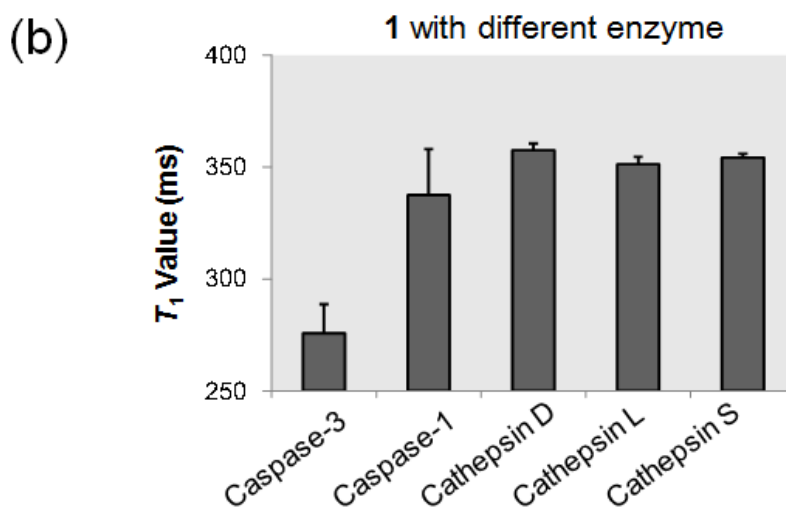
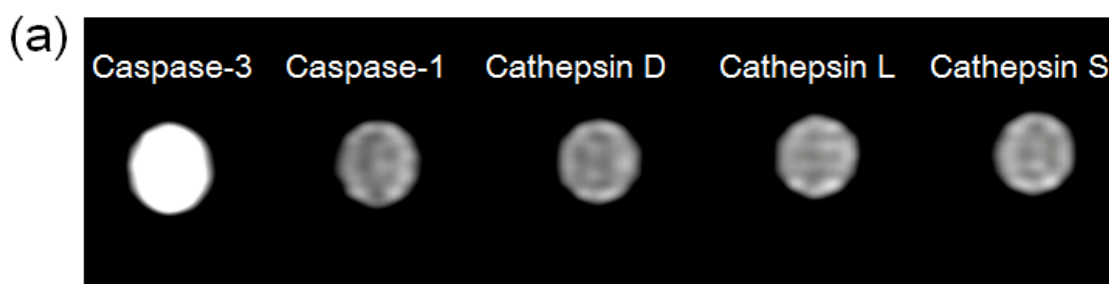


Fig. S6 The T_1 values (1 T) of the solution of **1** (208 μM) after incubation with 0, 1, 2.5, 5, 10, 25 and 50 nM caspase-3 in enzyme reaction buffer (pH 7.4) for 24 h. T_1 values were measured at 1 T scanner (Bruker Icon, Bruker BioSpin Corp.) at r.t., using a series of inversion-prepared fast spin-echo scans. Significantly shorter T_1 values were obtained after incubation with ≥ 5 nM caspase-3.

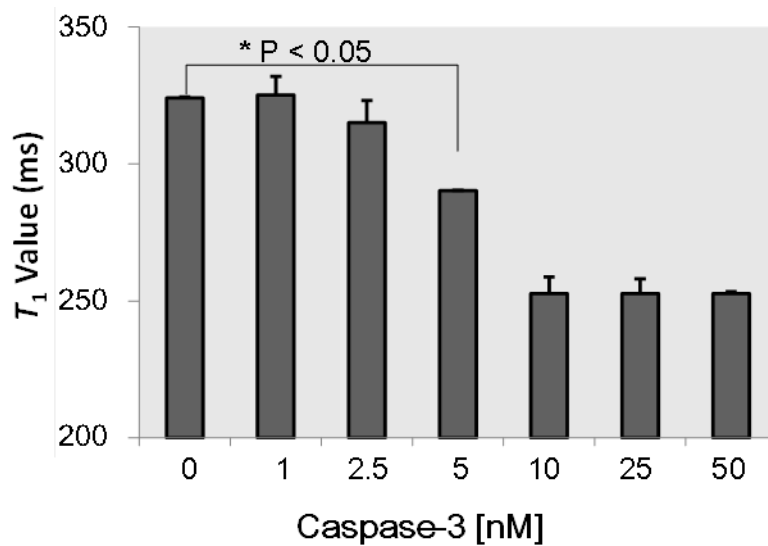


Fig. S7 Cytotoxicity study of **1** or Dotarem in cultured cells. HeLa Cells were incubated with 250 μ M **1** or Dotarem for 24 h. The cell numbers were counted using a Trypan Blue Assay, and normalized to that of cells without probe incubation. Error bars indicated standard deviation, coming from three independent experiments. No obvious cytotoxicity was observed in HeLa cells after incubation with 250 μ M **1** or Dotarem for 24 h.

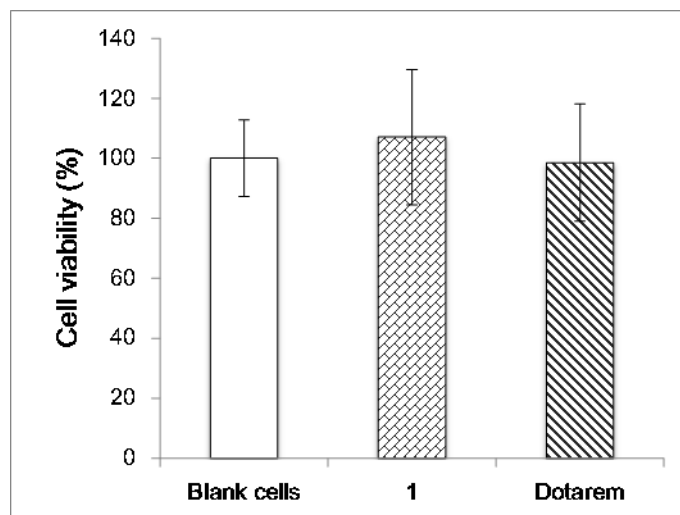


Fig. S8 STS-induce apoptosis and activation of caspase-3 in HeLa cells. (A) Measurement of caspase-3 activity in control and STS-treated HeLa cells. Cells were non-treated (control) or treated with 2 μM STS for 4 h and kept growing in blank culture medium without STS for another 24 h. Cells were then lysed, and the caspase-3 activity was measured using Caspase-Glo[®] 3/7 assay, and normalized to total protein mass. Each data point and error bar represents the mean and standard deviation of three experiments. (B) HPLC traces of incubation of **1** (50 μM) in viable and STS-induced apoptotic HeLa cell lysates overnight. HeLa cells (~ 8 millions) were either non-treated or treated with 2 μM STS for 4 h and kept growing in blank culture medium without STS for another 24 h. Cells were then lysed with RIPA buffer, and incubated with **1** (50 μM) followed by analyzing the reaction with HPLC assay (320 nm UV detection). Peaks * and ** indicate the disulfide reduction products; peaks # and ## indicate the cyclized products **2-I** and **2-II**.

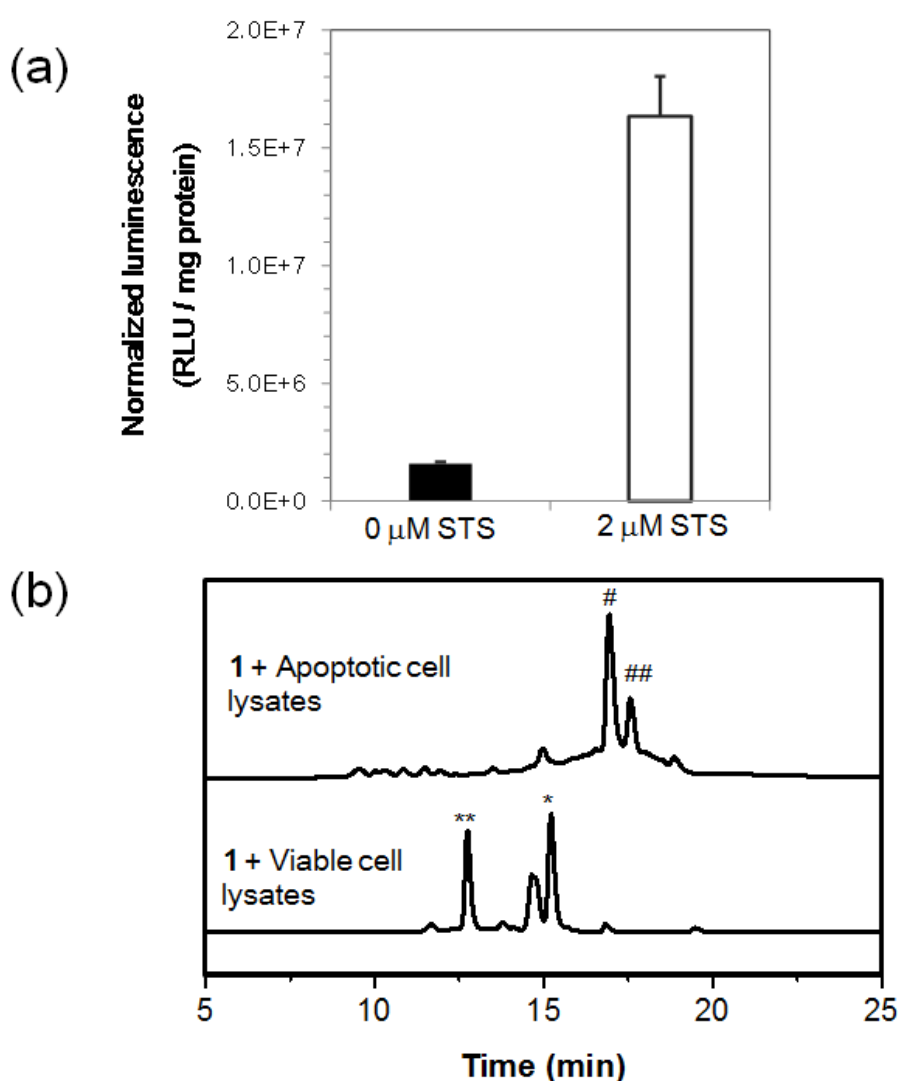


Fig. S9 Imaging of apoptotic cell with caspase-sensitive fluorescence probe **1-FITC**. (a) The chemical structure of **1-FITC** and proposed chemical reaction mediated by caspase-3/7 and reductant. **1-FITC** was designed to have a similar structure to **1**, but with a fluorophore **FITC** replacing Gd-chelate. Upon reduction and caspase-3/7 activation, **1-FITC** forms similar cyclized products and subsequent nano-aggregation, which is retained inside apoptotic cells and thus gives higher fluorescent signals relative to viable cells. (b) HPLC traces of **1-FITC** (black) and treatment of **1-FITC** (5 μ M) with recombinant human caspase-3 (25 nM) for 5 h at 37 $^{\circ}$ C in caspase-3 buffer (pH 7.4) (red). Peaks * and ** indicate the cyclized products **2-FITC**. (c) Fluorescence microscopy images of viable (STS (-)) and apoptotic (STS (+)) HeLa cells with **1-FITC**. Cells were non-treated (control) or treated with 2 μ M STS for 4 h, and then incubated with **1-FITC** (2 μ M) for another 24 h. Cell were stained with nuclear binding probe Hoechst 33342 (blue). Scale bar: 5 μ m.

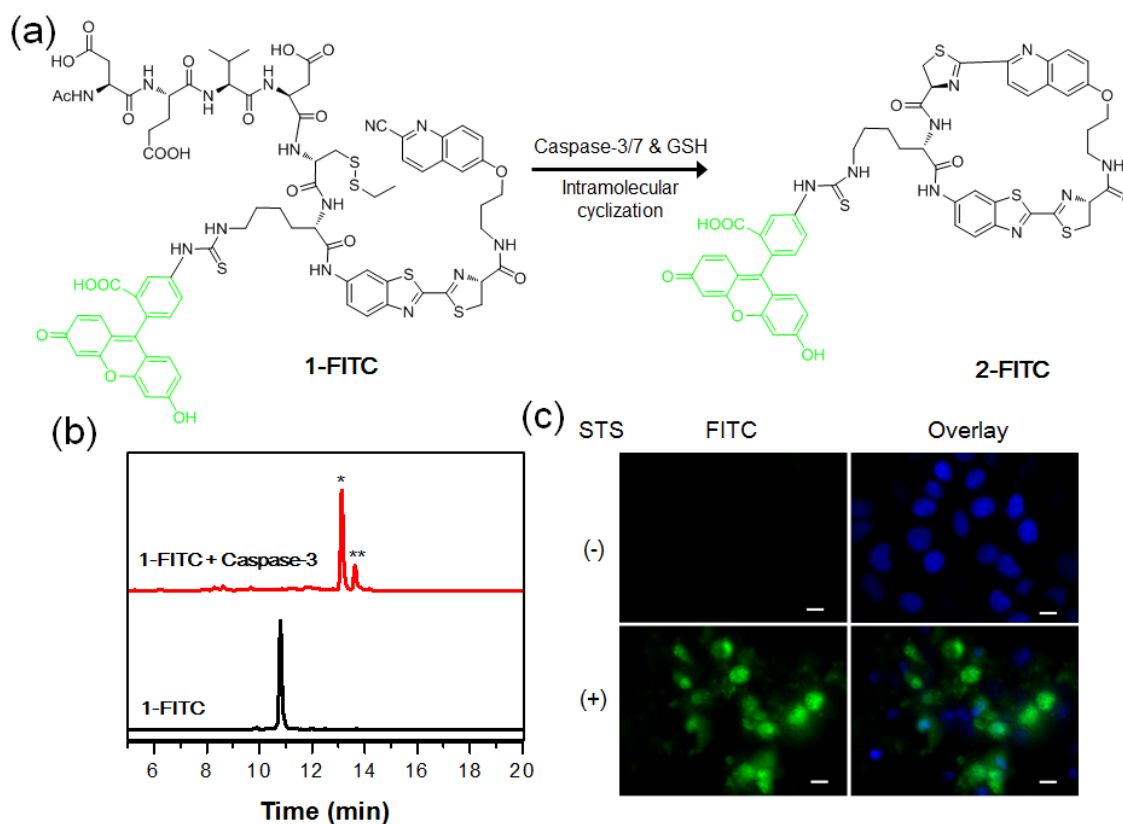
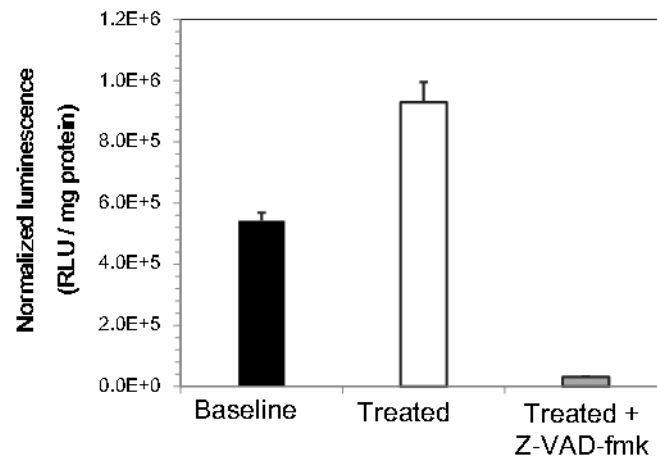


Fig. S10 Measurement of caspase-3 activity levels in non-treated (baseline) or DOX-treated tumor lysates. Mice bearing subcutaneous HeLa tumors were not treated or treated with intratumoral injection of two doses of 0.2 mg DOX. Two days after the last treatment, the mice were sacrificed. The tumors were taken out, and lysed with RIPA buffer (1 g/ml). The protein concentration in each lysate was measured by Bradford assay, and the caspase-3 activity was measured using Caspase-Glo[®] 3/7 assay, which was normalized to total protein mass. A significant (~1.6-fold) increase in caspase-3 activity was seen in DOX-treated tumors compared to baseline tumors, which was inhibited by caspase inhibitor Z-VAD-fmk.



Supplementary Table S1. HPLC condition for the analysis of **1** and **1-ctrl.**^a

Time (minute)	Flow (ml/min.)	H ₂ O (0.1% TFA)%	CH ₃ CN (0.1% TFA)%
0	1.0	80	20
3	1.0	80	20
25	1.0	10	90
35	1.0	10	90
40	1.0	80	20
50	1.0	80	20

^a HPLC was operated on the reversed-phase C₁₈ (Phenomenex, 5 μm, 4.6 x 250 mm) column.

Supplementary Table S2. HPLC condition for the purification of **1** and **1-ctrl.**^a

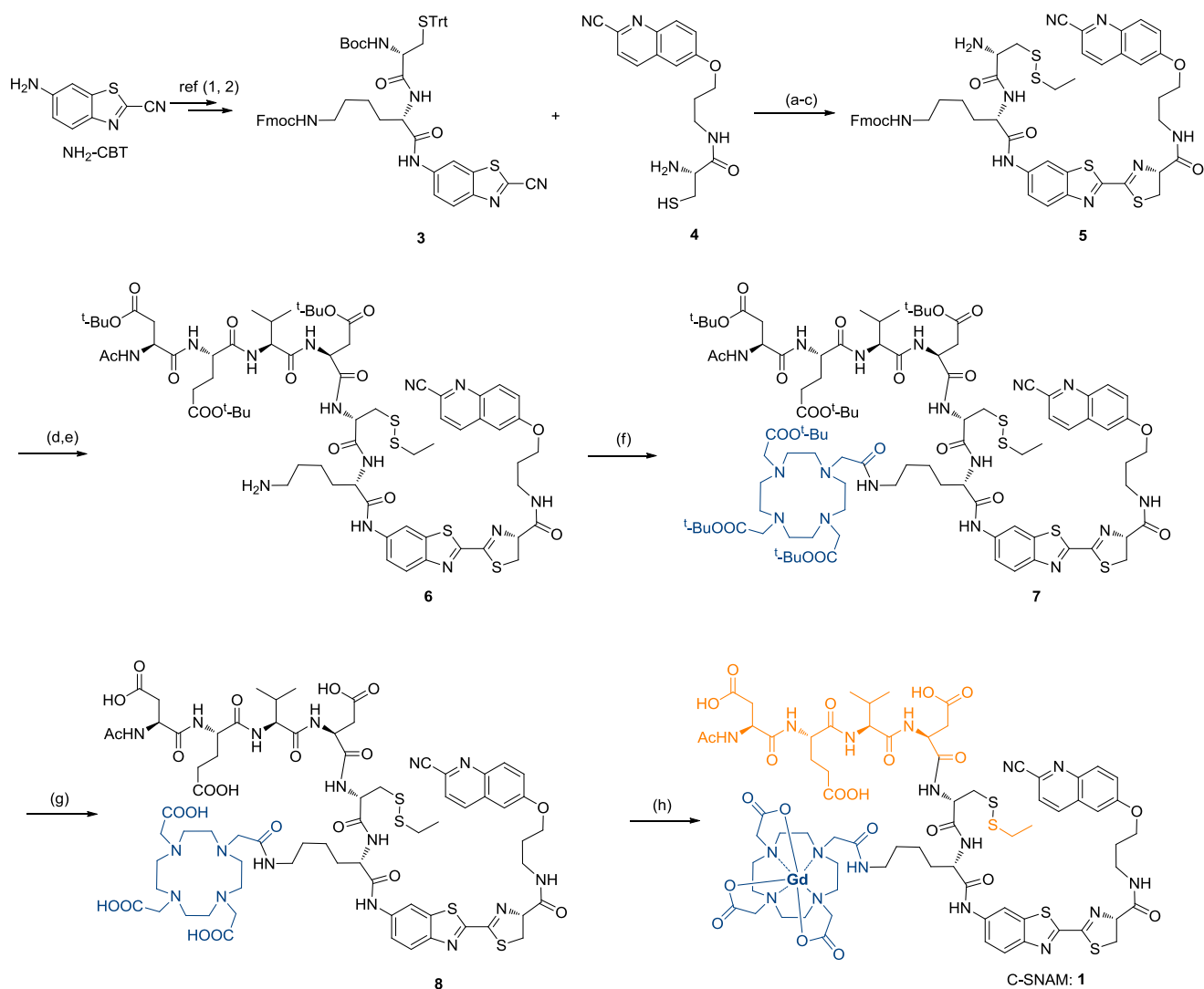
Time (minute)	Flow (ml/min.)	H ₂ O (0.1% TFA)%	CH ₃ CN (0.1% TFA)%
0	12.0	80	20
3	12.0	80	20
25	12.0	10	90
35	12.0	10	90
40	12.0	80	20

^a HPLC was operated on the reversed-phase C₁₈ (Phenomenex, 5 μm, 21.12 x 250 mm) column.

General methods

All chemicals were purchased from commercial sources (such as Aldrich, Anaspect and Chem-impex). Analytical TLC was performed with 0.25 mm silica gel 60F plates with fluorescent indicator (254 nm). Plates were visualized by ultraviolet light. The ^1H and ^{13}C NMR spectra were acquired on a Bruker 400 MHz magnetic resonance spectrometer. Data for ^1H NMR spectra are reported as follows: chemical shifts are reported as δ in units of parts per million (ppm) relative to chloroform-d (δ 7.26, s); multiplicities are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), or br (broadened); coupling constants are reported as a J value in Hertz (Hz); the number of protons (n) for a given resonance is indicated $n\text{H}$, and based on the spectral integration values. MALDI-MS and High Resolution Mass spectrometric analyses were performed at the Mass Spectrometry Facility of Stanford University. HPLC was performed on a Dionex HPLC System (Dionex Corporation) equipped with a GP50 gradient pump and an inline diode array UV-Vis detector. A reversed-phase C18 (Phenomenax, 5 μm , 4.6 \times 250 mm, 5 μm , or 21.2 \times 250 mm) column was used analysis and semi-preparation. UV absorbance of the probe was recorded on Agilent 8453 UV detector. TEM micrographs were obtained on a JEM 1230 Electron Microscope. Fluorescent microscopy images were acquired on an Olympus inverted fluorescence microscope (IX2-UCB) equipped with a Nuance multispectral imaging camera. MRI relaxivity was measured at 1T (Bruker Icon, Bruker BioSpin Corp.), 1.5 and 3 T (Signa HDx, GE Healthcare). Inductively coupled plasma mass spectrometry (ICP-MS) analysis was performed on Nu Plasma AttoM high-resolution ICP-MS.

Chemical synthesis and characterization of C-SNAF



Scheme S1. Synthesis of MRI probe C-SNAM (1). Reaction conditions: (a) TCEP, DIPEA, DCM/MeOH, Ar, r.t. 1 h; (b) 20% TFA/DCM; (c) PySSEt, MeOH; 31% for three steps; (d) Ac-Asp(O^tBu)-Glu(O^tBu)-Val-Asp(O^tBu)-COOH, HBTU, DIPEA, THF; (e) piperidine, DMF, 43% in two steps; (f) (t-BuO)₃DOTA-COOH, HBTU, DIPEA, DMF, 3 h; (g) TFA/TIPSH/DCM (95%/2.5%/2.5%); 56% in two steps; (h) $\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$, NaHCO_3 , pH 6-7, 75% for 1.

Synthesis of compound 3: Starting from $\text{NH}_2\text{-CBT}$, compound 1 was obtained as a white solid according to the methods reported previously [ref s1 and s2]. ¹H NMR (400 MHz, CDCl_3) δ 9.71 (s, 1H), 8.48 (s, 1H), 7.87 (d, $J = 9.0$ Hz, 1H), 7.64 (d, $J = 7.5$ Hz, 2H), 7.54 (m, 2H), 7.47 (d, $J = 7.2$ Hz, 2H), 7.35 – 7.23 (m, 8H), 7.22 – 7.05 (m, 11H), 4.44 (m, 1H), 4.25 (d, $J = 6.9$ Hz, 2H), 4.11 – 4.01 (m, 1H), 3.84 (m, 3H), 3.01 (m, 2H), 2.52 (m, 2H), 1.87 (s, br, 1H), 1.65 (m, 1H), 1.42 (m, 2H), 1.32 (s,

9H); ^{13}C NMR (101 MHz, CDCl_3) δ 171.87, 171.07, 157.30, 155.97, 148.56, 144.40, 144.01, 141.37, 139.00, 136.66, 135.18, 129.59, 128.20, 127.83, 127.18, 127.10, 125.13, 125.02, 121.40, 120.08, 113.20, 111.71, 80.70, 67.25, 66.71, 54.21, 54.12, 49.77, 49.56, 49.34, 49.13, 48.92, 48.70, 48.49, 40.33, 33.74, 31.39, 29.40, 28.70, 28.31, 22.68. MS: calcd. for $\text{C}_{56}\text{H}_{55}\text{N}_6\text{O}_6\text{S}_2^+$ [(M+H) $^+$]: 971.4; found MALDI-MS: m/z 971.

Synthesis of compound 5: To a solution of **3** (0.5 mmol), tris(2-carboxyethyl) phosphine hydrochloride (TCEP HCl, 0.5 mmol), and DIPEA (3.0 mmol) in 25 ml $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (1/1) under N_2 was added a solution of **2** (0.53 mmol) in MeOH dropwise at room temperature (r.t.), and the mixture was kept stirring at r.t. for further 30 min. After the reaction was completed, the solvent was removed under vacuum, and the residue was dissolved in ethyl acetate (EA, 30 ml). The solution was washed with water (2 \times 20 ml), brine, and dried with Na_2SO_4 . After removal of EA, the residue was directly dissolved in 20% TFA/ CH_2Cl_2 , and the mixture was kept stirring at r.t. for another 1 h to completely deprotect the Boc and Trt groups. The solvent was removed, and cold Et_2O (35 ml) was added to precipitate the intermediate. After centrifugation (2500 rpm) at 4 $^\circ\text{C}$, the precipitate was dissolved in MeOH, to which was added 2-(ethyldisulfanyl)pyridine (PySSEt, 0.55 mmol). After stirring at r.t. for 20 min, MeOH was removed, and the residue was further precipitated out with cold Et_2O (35 ml). Compound **5** was obtained as light yellow foam after purification by silica gel chromatography with an eluent of $\text{CH}_2\text{Cl}_2/\text{MeOH}$ (40/1 to 20/1). There was a combined yield of 31% for three steps. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 10.63 (s, 1H), 9.10 (d, $J = 7.5$ Hz, 1H), 8.54 (s, 1H), 8.41 (m, 4H), 8.31 (t, $J = 5.6$ Hz, 1H), 8.06 (d, $J = 8.9$ Hz, 1H), 7.97 (d, $J = 9.3$ Hz, 1H), 7.90 (d, $J = 8.5$ Hz, 1H), 7.84 (d, $J = 7.5$ Hz, 2H), 7.68 (d, $J = 9.1$ Hz, 1H), 7.63 (d, $J = 7.4$ Hz, 2H), 7.53 (dd, $J = 9.3, 2.6$ Hz, 1H), 7.44 (d, $J = 2.6$ Hz, 1H), 7.37 (t, $J = 7.4$ Hz, 2H), 7.28 (m, 3H), 5.32 (t, $J = 9.3$ Hz, 1H), 4.51 (dd, $J = 13.6, 7.4$ Hz, 1H), 4.24 (d, $J = 6.9$ Hz, 2H), 4.21 – 4.10 (m, 5H), 3.74 – 3.61 (m, 2H), 3.10 – 3.24 (m, 2H), 2.97 (m, 3H), 2.75 (q, $J = 8$ Hz, 2H), 2.08 – 1.96 (m, 2H), 1.70 (m, 3H), 1.41 (m, 3H), 1.25 (t, $J = 8$ Hz, 3H). MS: calcd. for $\text{C}_{50}\text{H}_{52}\text{N}_9\text{O}_6\text{S}_4^+$ [(M+H) $^+$]: 1002.3; found ESI-MS: m/z 1002.7.

Synthesis of 6: The peptide of Ac-Asp(O t Bu)-Glu(O t Bu)-Val-Asp(O t Bu)-COOH was prepared by solid phase peptide synthesis. To a solution of Ac-Asp(O t Bu)-Glu(O t Bu)-Val-Asp(O t Bu)-COOH (1.05 equiv.), HBTU (1.1 equiv.), and DIPEA (2.0 equiv.) in dry THF (30 ml), was added **5** (0.3 mmol), and the mixture was kept stirring at r.t. for 2~3 h. After the reaction was completed, THF was removed.

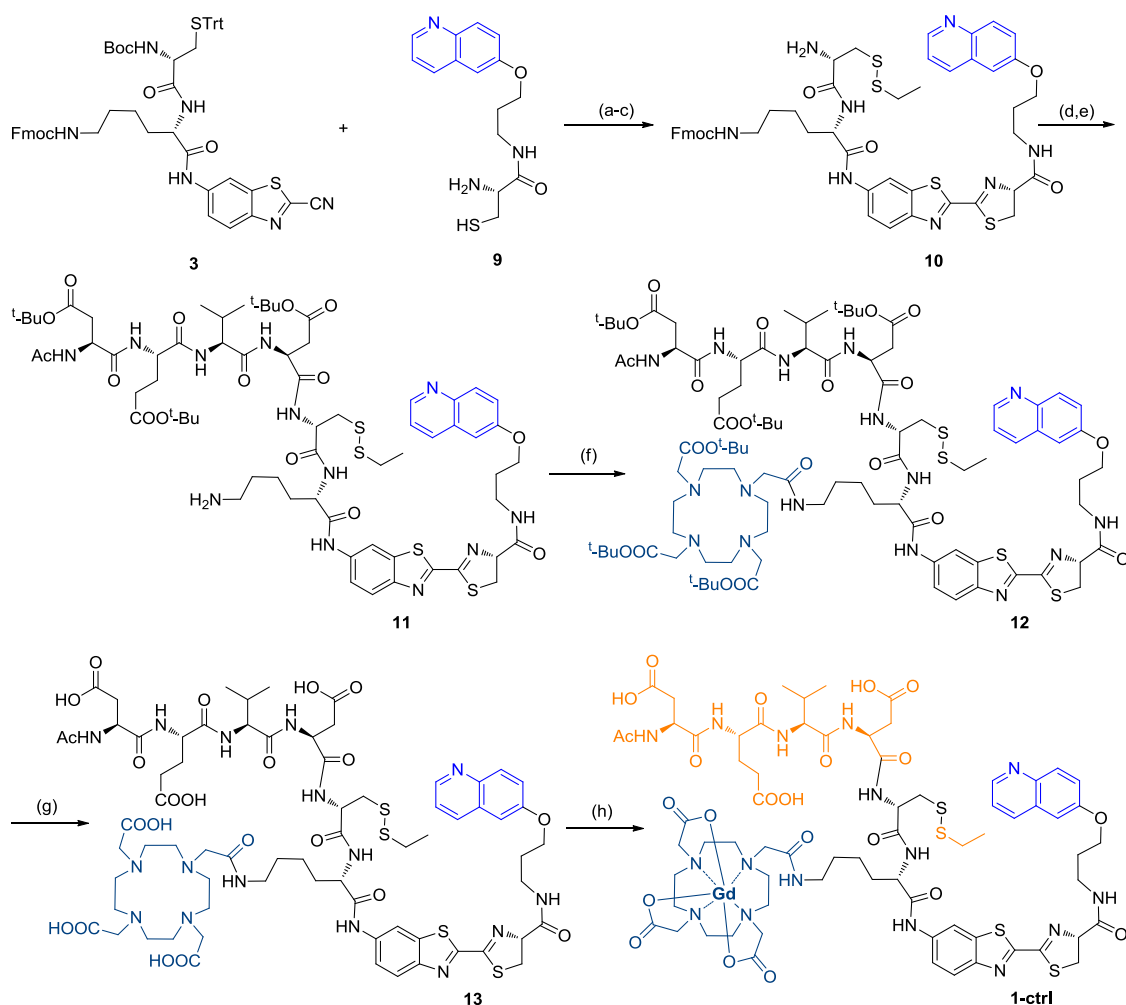
The residue was purified with a short silica gel column with the eluent of CH₂Cl₂/MeOH (15/1), which was followed by deprotection of the Fmoc-group with 5% piperidine in DMF at r.t. for 10 min. The reaction was quenched with 1 N HCl, and purified by HPLC to furnish compound **6** after lyophilization. A combined yield of 43% for two steps. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.42 (s, 1H), 8.60 (dd, *J* = 7.9, 1.8 Hz, 1H), 8.43 (d, *J* = 8.5 Hz, 2H), 8.31 (m, 2H), 8.19 (m, 2H), 8.06 (d, *J* = 8.9 Hz, 1H), 8.01 – 7.86 (m, 3H), 7.80 – 7.60 (m, 5H), 7.53 (dd, *J* = 9.3, 2.7 Hz, 1H), 7.45 (d, *J* = 2.1 Hz, 1H), 5.31 (t, *J* = 9.2 Hz, 1H), 4.62 – 4.47 (m, 3H), 4.42 (s, br, 1H), 4.32 (s, br, 1H), 4.19 (t, *J* = 6.1 Hz, 2H), 4.14 – 4.02 (m, 1H), 3.84 – 3.61 (m, 10H), 3.06 (m, 1H), 2.99 – 2.90 (m, 1H), 2.81 – 2.73 (m, 2H), 2.69 (m, 2H), 2.59 (dd, *J* = 15.9, 5.3 Hz, 1H), 2.44 – 2.35 (m, 1H), 2.13 (m, 2H), 2.02 (m, 2H), 1.92 – 1.79 (m, 5H), 1.67 (s, br, 2H), 1.54 (m, 2H), 1.46 – 1.26 (m, 29H), 1.21 (t, *J* = 8.0 Hz, 3H), 0.84 – 0.68 (m, 6H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 172.44, 171.66, 171.44, 171.29, 171.03, 170.20, 170.06, 169.96, 169.80, 164.89, 159.84, 159.38, 159.14, 158.81 (TFA), 149.33, 144.30, 138.74, 137.20, 136.96, 131.38, 131.00, 130.58, 125.38, 124.87, 124.79, 120.37, 118.60, 112.15, 106.86, 80.92, 80.77, 80.20, 79.96, 66.78, 63.74, 58.27, 54.06, 53.06, 52.46, 50.38, 50.13, 41.27, 39.35, 37.99, 36.71, 35.27, 32.26, 31.90, 31.28, 29.12, 28.31, 27.86, 27.30, 23.15, 23.02, 19.76, 18.72, 14.93. MS: calcd. for C₆₇H₉₄N₁₃O₁₅S₄⁺ [(M+H)⁺]: 1448.6; found MALDI-MS: *m/z* 1449.1.

Synthesis of 7: A mixture of (t-BuO)₃DOTA-COOH (0.11 mmol), HBTU (0.12 mmol), and DIPEA (0.21 mmol) in dry DMF (5 ml) was stirred at r.t. for 5 min, to which was added compound **6** (0.1 mmol). The reaction solution was kept stirring at r.t. for another 2 h. After the reaction was completed, the mixture was poured into water (40 ml). The precipitate was collected after centrifugation for 5 min (3000 rpm), which was further washed with water (30 ml) once, and dried after lyophilization. MS: calcd. for C₉₅H₁₄₄N₁₇O₂₂S₄ [(M+H)⁺]: 2003.0; found MALDI-MS: *m/z* 2002.9.

Synthesis of 8: Compound **7** from the last step was deprotected using a solution of TFA/ACN/TIPSH (95/2.5/2.5) at r.t. for 3 h. After the reaction, the solvent was removed under vacuum, and the residue was purified by HPLC to obtained **8** as light yellow powder after lyophilization. 56% yield from **6**. ¹H NMR (400 MHz, DMSO) δ 10.34 (s, 1H), 8.60 (d, *J* = 9.8 Hz, 1H), 8.41 (m, 3H), 8.34 – 8.24 (m, 2H), 8.20 (d, *J* = 7.7 Hz, 2H), 8.06 (d, *J* = 9.0 Hz, 1H), 7.98 (t, *J* = 7.8 Hz, 2H), 7.92 (dd, *J* = 8.5, 2.4 Hz, 1H), 7.67 (m, 2H), 7.54 (dd, *J* = 9.3, 2.7 Hz, 1H), 7.47 (d, *J* = 2.5 Hz, 1H), 5.30 (t, *J* = 9.3 Hz, 1H), 4.51 (m, 3H), 4.41 (m, 1H), 4.30 (m, 1H), 4.19 (t, *J* = 6.0 Hz, 2H), 4.07 (m, 3H), 3.86 (s, 2H), 3.76 –

3.49 (m, 16H), 3.16 – 2.91 (m, 12H), 2.79 – 2.63 (m, 4H), 2.63 – 2.52 (m, 2H), 2.18 (m, 2H), 2.02 (m, 2H), 1.97 – 1.86 (m, 2H), 1.85 – 1.62 (m, 6H), 1.37 (m, 4H), 1.21 (t, $J = 8$ Hz, 3H), 0.75 (m, 6H). MS: calcd. for $C_{71}H_{96}N_{17}O_{22}S_4^+$ [(M+H) $^+$]: 1666.6; found MALDI-MS: m/z 1667.5.

Synthesis of MR probe 1: To a solution of **8** in water was carefully added 0.5 M $NaHCO_3$ solution to adjust the pH value to 7, which was following by addition of a solution of $GdCl_3$ (3 – 5 equivalent to **8**) in water. After stirring at r.t. for 10 min, the pH value of the reaction solution was further adjusted to 7 using 0.5 M $NaHCO_3$ solution. The reaction mixture was bubbled with Ar, and then kept stirring at r.t. overnight. After centrifugation (2500 rpm, 3 min), the supernatant was purified by HPLC to obtain probe **1** as a light yellow powder after lyophilization. 75% yield. MALDI-MS: calcd. for $C_{71}H_{93}GdN_{17}O_{22}S_4^+$ [(M+H) $^+$]: 1821.5; found MALDI-MS: m/z 1821.4. HRMS: calcd. for $C_{71}H_{94}GdN_{17}O_{22}S_4^{2+}$ [(M+2H) $^{2+}$]: 1822.4883; found HR-ESI/MS: 911.2458 [(M+2H)/2] $^+$.



Scheme S2. Synthesis of **1-ctrl**. Reaction conditions: (a) TCEP, DIPEA, DCM/MeOH, Ar, r.t. 1 h; (b)

20% TFA/DCM; (c) PySSEt, MeOH; 47% yield for three steps; (d) Ac-Asp(O^tBu)-Glu(O^tBu)-Val-Asp(O^tBu)-COOH, HBTU, DIPEA, THF; (e) piperidine, DMF, 51% in two steps; (f) (t-BuO)₃DOTA-COOH, HBTU, DIPEA, DMF, 3 h; (g) TFA/TIPSH/DCM (95%/2.5%/2.5%); 53% in two steps; (h) GdCl₃·6H₂O, pH 6-7, 82%.

Synthesis of 10: A solution of **1** (0.5 mmol), **9** (0.53 mmol), TCEP HCl, (0.5 mmol), and DIPEA (2.2 mmol) in 20 ml CH₂Cl₂/MeOH (1/1) was stirring at room temperature for 30 min. After the reaction was completed, the solvent was removed under vacuum, and the residue was dissolved in ethyl acetate (EA, 30 ml). The solution was washed with water (2 × 20 ml), brine, and dried with Na₂SO₄. After removal of EA, the residue was purified with a short silica gel chromatography with eluent of CH₂Cl₂/MeOH (30/1 to 20/1) to afford crude intermediate, which was directly deprotected with 5 ml 20% TFA/CH₂Cl₂ (1% TIPSH) at r.t. for 1 h. After the reaction, the solvent was removed, and cold Et₂O (35 ml) was added to precipitate the intermediate. After centrifugation (2500 rpm), the precipitate was dissolved in MeOH, to which was added PySSEt (0.6 mmol), and continued reaction for another 20 min. After the reaction, MeOH was removed, and the residue was precipitated out with cold Et₂O (35 ml), followed by purified with silica gel chromatography with an eluent of CH₂Cl₂/MeOH (25/1 to 15/1) to afford compound **10** as light yellow foam. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.64 (s, 1H), 9.13 (d, *J* = 7.3 Hz, 1H), 8.94 (d, *J* = 4.3 Hz, 1H), 8.63 (d, *J* = 8.3 Hz, 1H), 8.54 (s, 1H), 8.44 (s, br, 2H), 8.34 (t, *J* = 5.5 Hz, 1H), 8.06 (dd, *J* = 9.0, 2.3 Hz, 2H), 7.83 (d, *J* = 7.5 Hz, 2H), 7.75 (m, 1H), 7.62 (m, 4H), 7.53 (d, *J* = 2.5 Hz, 1H), 7.36 (t, *J* = 7.4 Hz, 2H), 7.28 (m 3H), 5.33 (t, *J* = 9.2 Hz, 1H), 4.51 (m, 1H), 4.31 – 4.07 (m, 6H), 3.79 – 3.60 (m, 2H), 3.18 (m, 2H), 2.98 (m, 2H), 2.74 (m, 2H), 2.13 – 1.95 (m, 2H), 1.75 (m, 2H), 1.54 – 1.24 (m, 6H), 1.24 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (101 MHz, DMSO) δ 171.30, 169.80, 167.60, 164.92, 159.88, 159.52, 159.19 (TFA), 158.29, 156.76, 149.38, 145.20, 144.55, 141.37, 141.03, 138.71, 138.46, 136.95, 130.47, 128.23, 127.67, 126.34, 125.92, 125.76, 124.91, 122.69, 120.76, 120.41, 112.24, 107.49, 79.96, 66.73, 65.87, 54.63, 51.86, 47.41, 39.54, 36.72, 35.30, 32.34, 31.93, 29.74, 29.14, 23.34, 14.76. MS: calcd. for C₄₉H₅₃N₈O₆S₄⁺ [(M+H)⁺]: 977.3; found ESI-MS: m/z 978.1.

Synthesis of 11: A solution of **7** (0.3 mmol), Ac-Asp(O^tBu)-Glu(O^tBu)-Val-Asp(O^tBu)-COOH (1.05 equiv.), HBTU (1.1 equiv.), and DIPEA (2.0 equiv.) in dry THF (20 ml) was kept stirring at r.t. for 2~3 h. After the reaction was completed, THF was removed. The residue was purified with a short silica

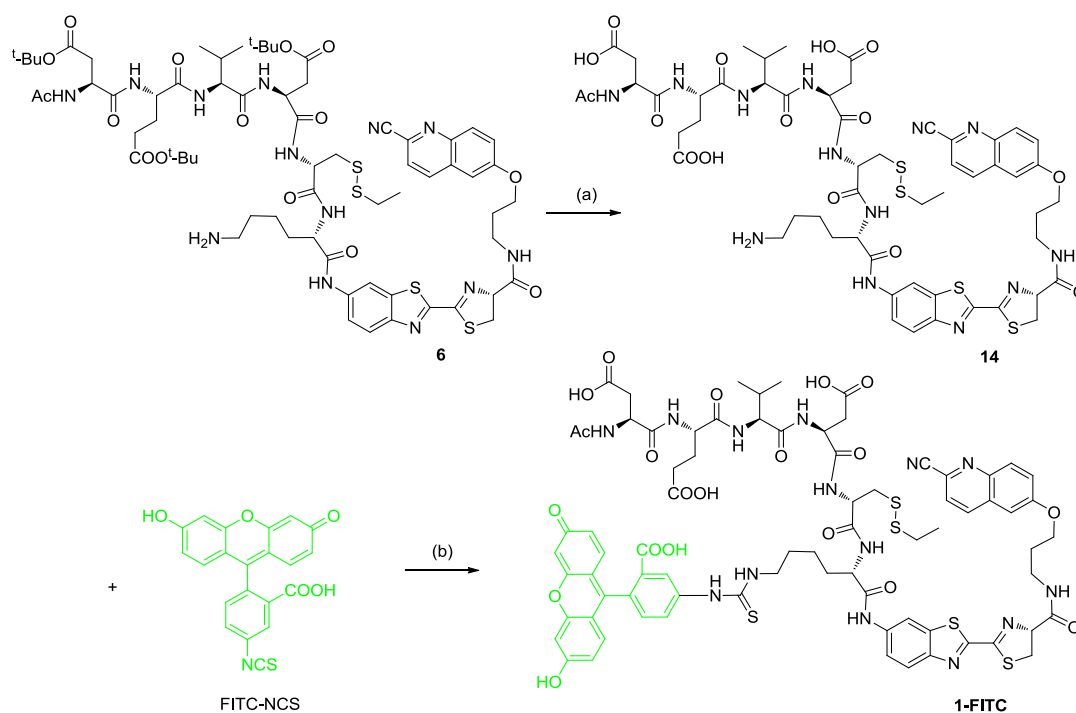
gel column with the eluent of CH₂Cl₂/MeOH (15/1), which was followed by deprotection of the Fmoc-group with 5% piperidine in DMF at r.t. for 10 min. The reaction was quenched with 1 N HCl, and purified by HPLC to furnish compound **11** after lyophilization (51% yield in two steps). ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.43 (s, 1H), 8.90 (d, *J* = 4.7 Hz, 1H), 8.64 – 8.52 (m, 2H), 8.46 (m, 1H), 8.31 (m, 2H), 8.21 (m, 2H), 8.10 – 7.91 (m, 3H), 7.89 – 7.62 (m, 6H), 7.56 (dd, *J* = 9.2, 2.5 Hz, 1H), 7.51 (d, *J* = 2.3 Hz, 1H), 5.31 (t, *J* = 9.2 Hz, 1H), 4.54 (m, 3H), 4.42 (s, br, 1H), 4.34 (m, 1H), 4.17 (t, *J* = 6.1 Hz, 2H), 4.13 – 4.06 (m, 1H), 3.78 – 3.58 (m, 2H), 3.47 (t, *J* = 5.3 Hz, 1H), 3.15 – 3.03 (m, 1H), 2.95 (m, 1H), 2.84 – 2.64 (m, 5H), 2.60 (dd, *J* = 15.8, 5.1 Hz, 1H), 2.48 – 2.35 (m, 2H), 2.24 – 2.08 (m, 2H), 2.02 (m, 2H), 1.95 – 1.76 (m, 6H), 1.70 (s, br, 2H), 1.55 (m, 2H), 1.50 – 1.26 (m, 30H), 1.25 – 1.17 (m, 3H), 0.75 (m, 6H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 172.44, 171.67, 171.46, 171.30, 171.04, 170.23, 170.08, 169.96, 169.81, 164.89, 159.82, 159.77, 159.44, 159.10, 158.76 (TFA), 158.11, 149.32, 145.79, 140.09, 139.48, 138.76, 136.96, 130.35, 127.15, 125.41, 124.86, 122.63, 120.38, 118.59, 115.66, 112.16, 107.45, 80.98, 80.91, 80.80, 80.76, 80.18, 79.96, 72.95, 66.69, 60.93, 58.31, 54.10, 53.09, 52.49, 50.41, 50.25, 50.17, 41.29, 39.33, 37.99, 37.88, 36.73, 35.28, 32.27, 31.91, 31.80, 31.27, 29.17, 28.33, 28.30, 27.85, 27.29, 23.13, 23.04, 19.76, 18.72, 14.91. MS: calcd. for C₆₆H₉₅N₁₂O₁₅S₄⁺ [(M+H)⁺]: 1423.6; found MALDI-MS: m/z 1424.2.

Synthesis of 12: A mixture of (t-BuO)₃DOTA-COOH (0.11 mmol), HBTU (0.12 mmol), and DIPEA (0.21 mmol) in dry DMF (5 ml) was stirred at r.t. for 5 min, to which was added compound **11** (0.1 mmol). The reaction solution was kept stirring at r.t. for another 2 h. After the reaction was completed, the mixture was poured into water (40 ml). The precipitate was collected after centrifugation for 5 min (3000 rpm), which was further dried after lyophilization. MS: calcd. For C₉₄H₁₄₅N₁₆O₂₂S₄⁺ [(M+H)⁺]: 1978.0; found MALDI-MS: m/z 1978.1.

Synthesis of 13: Compound **12** was deprotected using a solution of TFA/DCM/TIPSH (95/2.5/2.5) at r.t. for 3 h. After the reaction, the solvent was removed under vacuum, and the residue was purified by HPLC to obtained **13** after lyophilization. 53% yield from **6**. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.35 (s, 1H), 9.01 (d, *J* = 4.2 Hz, 1H), 8.80 (d, *J* = 8.4 Hz, 1H), 8.58 (s, 1H), 8.48 (s, 1H), 8.39 (s, 1H), 8.34 – 8.16 (m, 3H), 8.11 (d, *J* = 8.3 Hz, 1H), 8.03 (m, 2H), 7.87 (dd, *J* = 8.1, 5.2 Hz, 1H), 7.77 – 7.41 (m, 5H), 5.31 (t, *J* = 9.2 Hz, 1H), 4.51 – 4.62 (m, 3H), 4.39 (s, 1H), 4.32 (s, 1H), 4.19 (m, 2H), 4.09 (m, 3H), 3.91 (s, 2H), 3.79 – 3.23 (m, 16H), 3.04 (m, 12H), 2.82 – 2.50 (m, 6H), 2.19 (m, 2H), 2.00 (m,

4H), 1.76 (m, 6H), 1.38 (m, 4H), 1.21 (t, $J = 7.3$ Hz, 3H), 0.78 (m, 6H). ^{13}C NMR (101 MHz, DMSO- d_6) δ 174.79, 172.56, 172.40, 171.90, 171.80, 171.72, 171.56, 171.47, 170.60, 170.36, 170.30, 169.83, 164.92, 159.84, 159.22 (TFA, $J = 35$ Hz), 158.73, 149.31, 144.16, 142.94, 138.75, 136.97, 136.52, 130.75, 126.96, 124.85, 122.84, 120.40, 116.65 (TFA, $J = 294$ Hz), 112.16, 107.70, 79.93, 72.93, 66.88, 58.43, 55.35, 54.62, 54.32, 53.18, 53.14, 52.71, 51.24, 50.48, 50.35, 48.91, 48.54, 36.67, 35.28, 32.30, 31.91, 31.06, 30.74, 29.07, 27.70, 27.52, 23.61, 23.13, 19.84, 19.75, 18.65, 18.47, 17.63, 14.92. MS: calcd. for $\text{C}_{70}\text{H}_{97}\text{N}_{16}\text{O}_{22}\text{S}_4^+$ [(M+H) $^+$]: 1641.6; found MALDI-MS: m/z 1641.8.

Synthesis of 1-ctrl: A NaHCO_3 solution (0.5 M) was carefully added to a solution of **13** in water to adjust the pH value to 7, which was following by addition of a solution of GdCl_3 (3 – 5 equivalent to **13**) in water. After stirring at r.t. for 10 min, the pH value of the reaction solution was further adjusted to 7 using 0.5 M NaHCO_3 solution. The reaction mixture was bubbled with Ar, and then kept stirring at r.t. overnight. After centrifugation (2500 rpm, 3 min), the supernatant was purified by HPLC to obtain probe **1** as light yellow powder after lyophilization. 82% yield. MALDI-MS: calcd. for $\text{C}_{70}\text{H}_{94}\text{GdN}_{16}\text{O}_{22}\text{S}_4^+$ [(M+H) $^+$]: 1796.5; found MALDI-MS: m/z 1796.9. HRMS: calcd. for $\text{C}_{70}\text{H}_{94}\text{GdN}_{16}\text{O}_{22}\text{S}_4^+$ [(M+H) $^+$]: 1796.4856; found HR-ESI/MS: 1796.4864.



Scheme S3. Synthesis of **1-FITC**. Reaction conditions: (a) TFA/TIPSH/DCM (95%/2.5%/2.5%), 81%; (b) DIPEA, DMF.

Synthesis of 14: Compound **6** was deprotected using a solution of TFA/CH₃CN/TIPSH (95/2.5/2.5) at r.t. for 3 h. After the solvent was removed, cold Et₂O was added, and the precipitate was dried under vacuum to give compound **5** in 83% yield without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 10.37 (s, 1H), 8.59 (d, *J* = 9.7 Hz, 1H), 8.43 (d, *J* = 8.0 Hz, 2H), 8.36 – 8.13 (m, 4H), 8.12 – 7.86 (m, 5H), 7.66 (m, 5H), 7.53 (d, *J* = 9.2 Hz, 1H), 7.46 (s, 1H), 5.30 (t, *J* = 9.0 Hz, 1H), 4.66 – 4.27 (m, 5H), 4.18 (s, br, 2H), 4.13 – 4.04 (m, 1H), 3.36 (m, 3H), 3.15 – 3.02 (m, 1H), 2.96 (m, 1H), 2.85 – 2.64 (m, 5H), 2.56 (m, 2H), 2.44 (m, 1H), 2.16 (m, 2H), 2.09 – 1.63 (m, 11H), 1.54 (s, br, 2H), 1.37 (m, 2H), 1.21 (t, *J* = 6.9 Hz, 3H), 0.77 (m, 6H); ¹³C NMR (101 MHz, DMSO) δ 174.81, 172.40, 172.36, 171.82, 171.70, 171.42, 170.31, 169.81, 164.92, 159.87, 159.39, 149.32, 144.30, 138.72, 137.22, 136.99, 131.39, 131.01, 130.58, 125.40, 124.89, 124.81, 120.39, 118.62, 112.18, 106.87, 79.94, 66.76, 58.39, 54.10, 53.19, 52.65, 50.48, 50.29, 39.38, 36.70, 36.64, 36.48, 35.30, 32.28, 31.75, 31.11, 30.73, 29.11, 27.51, 27.30, 23.17, 23.05, 19.76, 18.71, 14.96. MS: calcd. for C₅₅H₇₀N₁₃O₁₅S₄⁺ [(M+H)⁺]: 1280.4; found MALDI-MS: m/z 1280.8.

Synthesis of 1-FITC: To a solution of **14** (5 mg) in dry DMF (0.5 ml) was added FITC-NCS (1.2 mg) and DIPEA (5 μl), and the mixture was kept stirring at r.t. for further 1 h. After the reaction was completed, the mixture was purified by HPLC to afford **1-FITC** in 57% yield. MS: calcd. For C₇₆H₈₁N₁₄O₂₀S₅⁺ [(M+H)⁺]: 1670.8; found MALDI-MS: 1672.6.

***In vitro* HPLC assay of enzymatic reaction.**

The enzymatic reaction for human recombinant caspase-3 (Sigma) were conducted in enzyme reaction buffer with 50 mM HEPES, 100 mM NaCl, 1 mM EDTA, 10 mM TCEP, 10% glycerol, and 0.1% CHAPS at pH 7.4. **1** (25 μM, 1 ml) was incubated in enzyme reaction buffer at r.t. for 10 min, to which caspase (50 nM) was added. Reactions were performed at 37 °C, and monitored by HPLC at 0.5, 1, 2, 3, 4, and 5 hours. The percentage conversion of **1** to **2** following the enzymatic reaction was calculated according to the percentage of peak area of **2** (**2-I** and **2-II**) in the HPLC trace at 320 nm UV detection.

MR Imaging Protocol for *r*1 relaxivity measurement

A series of solutions containing contrast agents with four to five different concentrations (0.05 – 0.5 mM) in caspase buffer (pH 7.4) were treated with or without caspase-3 (50 nM) at 37 °C overnight.

The solutions were then placed in PCR tubes, bundled together, and imaged at 1T (Bruker Icon, Bruker BioSpin Corp.), 1.5T and 3T (Signa HDx, GE Healthcare) at r.t. or 37 °C. The scanning procedure began with a localizer and then consisted of a series of inversion-prepared fast spin-echo scans, identical in all aspects (TR 8000 ms for GE, TR 6000 ms for Bruker Icon, TE minimum, field of view 6 cm, slice thickness 2 mm, matrix 128 x 128, NEX 1) except for the inversion time (TI) which was varied as follows: 4000, 2400, 1200, 800, 600, 400, 300, 200, 100, and 50 ms. This scanning procedure produced enough data to fit quantitative T₁ relaxation values for each sample in the image. For quantitative data analysis, the acquired MR images were transferred as DICOM images to an offline workstation, and signal intensities were extracted from each of the 5 samples at each of the 10 TI times by manual region of interest (ROI) placement and voxel averaging within the ROI.

Signal intensity versus TI relationships were fit to the following exponential T₁ recovery model by non-linear least squares regression: $SI(TI) = S_0 [1 - 2 \cdot \exp(-TI/T_1) + \exp(-TR/T_1)]$. Relaxation rates (R₁) were determined as 1/T₁. Longitudinal relaxivities (r_1 , units of mM⁻¹s⁻¹) were calculated as the slope of R₁ vs [Gd] after determination of true Gd concentration for each sample by the ICP-MS measurement.

Cell culture.

HeLa human cervical adenocarcinoma epithelial cells from the ATCC were cultured in Dulbecco's modified eagle medium (GIBCO) supplemented with 10% fetal bovine serum (FBS, GIBCO), 100 U/mL penicillin and 100 µg/mL streptomycin (GIBCO). For epifluorescence microscopy experiments, cells were plated onto #1.5 borosilicate 8-well chambered coverslips (Nunc) and grown to ~ 70% confluence. For cell uptake study, HeLa cells were plated onto 12-well clear plates and grown to ~ 70% confluence. For MR imaging of cell pellets experiment, HeLa cells were cultured in cell culturing dishes (75 mm, BD Falcon), and grown to ~ 70% confluence.

Cell viability study.

~ 200,000 HeLa cells in 1 mL DMEM medium were seeded onto 12-well plates. The following day, the medium was replaced with 1 ml of fresh medium, or medium containing **1** or Dotarem (250 µM). After 24 h incubation, the medium was removed, and the cells were trypsinized. The cell number was counted using trypan blue assay. The viability of cell with blank medium was set as 100%, and cells incubating with **1** or Dotarem were normalized to that in blank medium.

Caspase-Glo[®] 3/7 assays to measure the caspase-3/7 activity in drug-induced apoptotic cells.

HeLa cells (~ 4 million) were seeded onto cell culture dishes. After cell growth to ~ 70% confluence, medium was removed and 8 ml of fresh medium containing different concentration of STS (0, or 2 μ M) was added to the dishes and incubated for 4 h. Then, the medium was replaced with 8 ml of fresh medium with further incubation for 24 h. The medium was carefully removed, trypsinized, centrifuged (1200 rpm), and washed with PBS twice. The obtained cell pellets were lysed with RIPA buffer (200 μ l per dish), and the protein concentration was measured using Bradford assay with BSA as standard. The caspase-3/7 activity in the lysates were measured using the Caspase-Glo[®] 3/7 assay (Promega) according to the standard protocol. In general, cell lysates were diluted (1/1, v/v) in 100 μ L of caspase reaction buffer (2 \times , 100 mM HEPES, 200 mM NaCl, 2 mM EDTA, 20 mM TCEP, 20% glycerol, and 0.2% CHAPS, pH 7.4), to which 100 μ L of Caspase-Glo[®] 3/7 reagent was added to each solution. The mixture was gently mixed, and incubated in the dark at r.t. for 1 h, followed by measuring luminescence (RLU) with a luminometer (Turner Biosystems) as directed by the manufacturer. Data were then background subtracted, normalized by protein mass, and the caspase-3/7 activity was expressed as normalized luminescence per mg of protein.

Caspase-Glo[®] 3/7 assays to measure the caspase-3/7 activity in saline- and DOX-treated tumor lysates.

HeLa tumor xenografted mice were not treated or treated with DOX (0.2 mg \times 2) as described. After treatment, the mice were sacrificed, and the tumors were resected and lysed with RIPA buffer (1g tissue / 1 ml buffer). The solution was homogenized for 1 min on ice, three times. The lysed solution was kept on ice for another 30 min, and centrifuged at 14,000 \times g for 30 min at 4 $^{\circ}$ C. The supernatant was collected, and the protein concentration was measured using Bradford assay with BSA as standard. The caspase-3/7 activity in the lysed solution was measured by Caspase-Glo[®] 3/7 assay, and normalized to the protein mass.

Analysis of the reaction of 1 in viable and apoptotic cell lysates.

Adherent HeLa cells (~ 8 million) were either not treated or treated with STS (2 μ M) for 4 h. After replacing the medium containing STS with blank medium, the cells were kept growing for another 24 h. The culture medium was carefully removed and the cells were washed with cold PBS (1 \times). The

cells were trypsinized to form cell pellets, and RIPA (RadioImmuno Precipitation Assay, sigma) buffer was added to cells using 200 μ L of buffer per 4 million cells. Then the cells were kept on ice for 0.5 h. The cell lysate was gathered, centrifuged at 14,000 \times g for 10 min at 4 $^{\circ}$ C. The supernatant of the cell lysate was collected, and the caspase-3/7 activity in the lysate was determined by Caspase-Glo[®] 3/7 assay. The cell lysate was diluted with caspase reaction buffer (2 \times , 100 mM HEPES, 200 mM NaCl, 2 mM EDTA, 20 mM TCEP, 20% glycerol, and 0.2% CHAPS, pH 7.4) (1/1, v/v), and incubated with **1** (50 μ M) at 37 $^{\circ}$ C overnight and then injected into an HPLC system (Dionex) for analysis.

Epifluorescence microscopy imaging of STS-treated HeLa cells with 1-FITC.

HeLa cells in #1.5 borosilicate 8-well chambered coverslips (Nunc) were either not treated or treated with 2 μ M STS for 4 h. After removal of STS, cells were washed with fresh medium once. Fresh culture medium containing **1-FITC** (2 μ M) was added, and cells were incubated for 24 h. Then the medium was removed, the cells were carefully washed with PBS (1 \times) three times, and stained with nuclear binding probe Hoechst 33342 (2 μ M) at 37 $^{\circ}$ C for 30 min. After several rinses with PBS (1 \times), the medium was replaced and the fluorescence images were acquired with DAPI and FITC filters.

Cellular uptake study with contrast agents.

~ 200,000 HeLa cells in 1 mL DMEM medium were seeded onto 12-well plates. The following day, the cells were either not treated or treated with 2 μ M STS for 4 h. The medium was then replaced with 1 ml of fresh medium, or medium containing probe **1** (50, 100 and 250 μ M), Dotarem (250 μ M), **1-ctrl** (250 μ M), or **1** (250 μ M) together with Z-VAD-fmk (50 μ M). After 24 h incubation, the medium was removed, and the cells were trypsinized. The cell pellets were carefully washed with cold PBS (1 \times) three times, and then lysed with 2% SDS (PBS, 1 \times). The protein concentrations in the lysates were measured using a Bradford assay with BSA as standard. The whole cell lysates together with cell debris were then digested with 69% HNO₃ at 110 $^{\circ}$ C overnight. The residue was diluted in 2% HNO₃ solution, and the amount of Gd in each solution was measured by ICP-MS. The cellular uptake of Gd was normalized to protein mass.

MR imaging of HeLa cell pellets after incubation with 1 or Dotarem.

HeLa cells (~ 8 million) were seeded onto cell culture dishes. The next day, medium was removed and 8 ml of fresh medium containing different concentrations of STS (0, or 2 μ M) was added and incubated for 4 h. The medium was then replaced with 8 ml of fresh medium, or medium containing probe **1** (250 μ M), or Dotarem (250 μ M) with further incubation for 24 h. The medium was carefully removed, trypsinized, centrifuged (1200 rpm), and washed with PBS three times. The cell pellets were then fixed with 4% PFA, and put in PCR tubes, bundled together, and imaged at 1T (Bruker Icon, Bruker BioSpin Corp.) and 3T (Signa HDx, GE Healthcare) at r.t.. The T_1 values for cell pellets were acquired by a series of inversion-prepared fast spin-echo scans. T_1 -weighted spin-echo images (TE/TR = 30/100 ms) of cell pellets were acquired at 3T.

Chemotherapy mouse model.

Animal care and euthanasia were done with the approval of the Administrative Panels on Laboratory Animal Care of Stanford University. To establish tumors in 6-week-old female nu/nu mice, 2-million HeLa cells suspended in 50 μ L of a 50% v/v mixture of Matrigel in supplemented DMEM (10% FBS, 1% penstrep) were injected subcutaneously in the shoulders of the mouse. Tumors were grown until a single aspect was 0.7 to 0.9 cm (approx. 10-15 days), and then treatment, consisting of intratumoral injections of two doses of 0.2 mg doxorubicin separated by 2 days, was carried out.

***In Vivo* MR Imaging**

In vivo MR imaging was performed on an Aspect M2 1T permanent magnet (Aspect Imaging, Shoham, Israel) using a 40 mm Transmit/Receive mouse body coil. During imaging, the animals were anaesthetized with Isoflurane and their respiration and body temperature were constantly monitored. Intravenous contrast injection was administered in a 100 μ L volume at 0.1 mmol/kg concentration for every imaging session. Multi-slice (312 μ m in-plane, 12 slices with 1 mm thickness) T_1 -weighted spin echo images (TE/TR = 8.9/250 ms, matrix 128x128, FOV = 40 mm, NEX = 6) were acquired, one before contrast injection and then every 4 minutes up to 4 hours after contrast injection. A 1 mM Dotarem reference tube was inserted in the field of view to enable inter-session variability correction during post-processing. Each animal underwent two imaging session; one prior to DOX treatment (baseline), and one after DOX treatment (treated).

Analysis of MRI data

Acquired MRI data were transferred as DICOM images to an offline workstation and Image J (National Institute of Health) was used for post-processing and quantitative image analysis. This consisted of manual segmentation of the tumor ROI for each slice, slice-wise normalization of mean tumor signal intensity with the 1 mM Dotarem reference standard to account for inter-session variability, followed by combining these normalized, slice-wise values to generate mean volumetric tumor signal intensities (SI) for each time point.

% Signal enhancement (% SE): % Signal enhancement was calculated at each time point as the % difference between the tumor SI at that time point and the tumor SI in the pre-contrast (t=0) dataset.

$\% SE(t) = (SI(t) - SI(t=0))/SI(t=0)$; for each mouse, for every session

Statistical Analysis

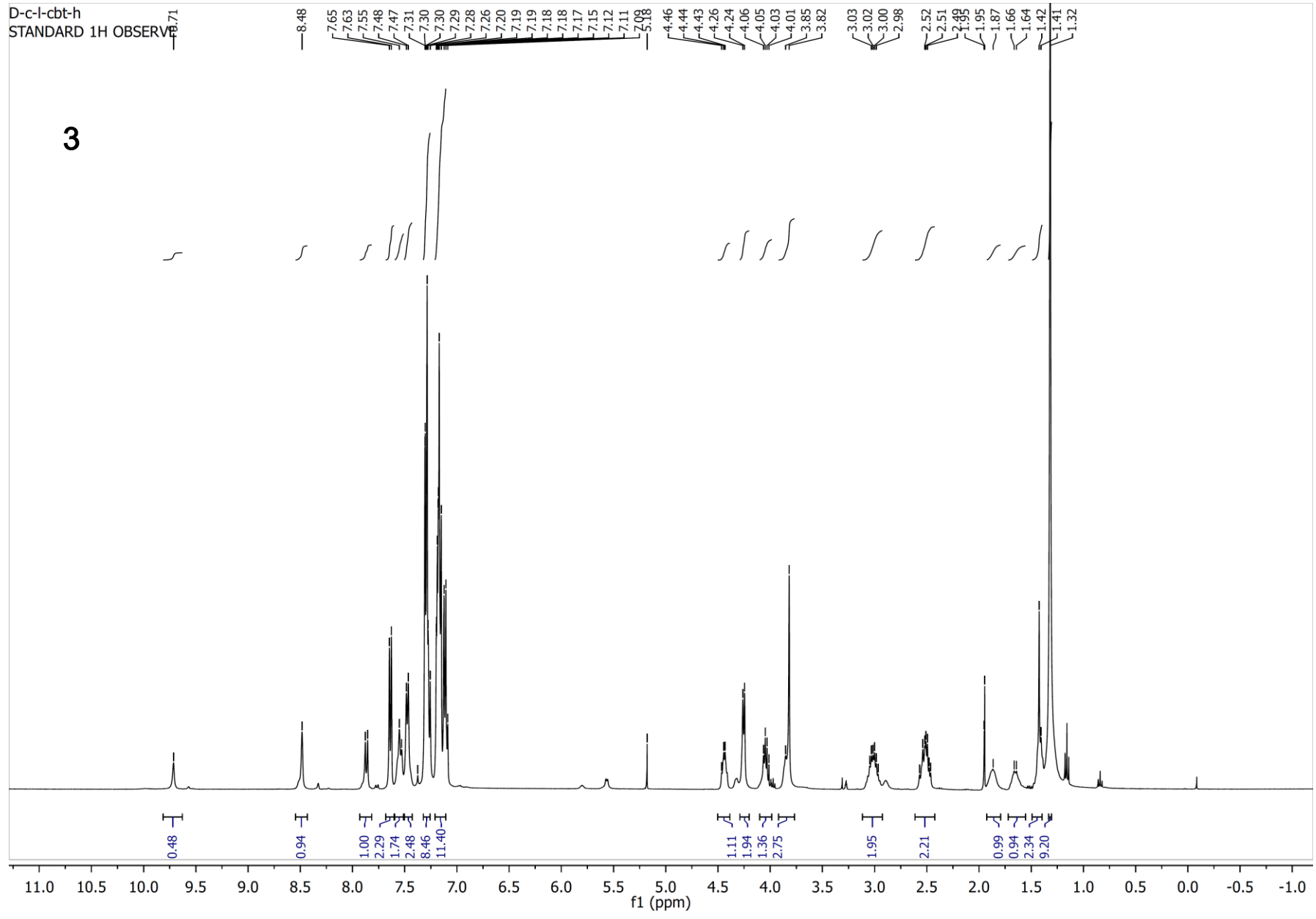
Results are expressed as the mean \pm standard deviation unless otherwise stated. Statistical comparisons between two groups were determined by t-test, and between 3 or more groups by one-way ANOVA followed by a post-hoc Tukey's HSD test. Time course analysis between groups was performed using general linear model repeated-measures analysis. Correlation analyses were performed using one-tailed Spearman r (non-parametric). For all tests, $p < 0.05$ was considered statistically significant. All statistical calculations were performed using GraphPad Prism v. 5 (GraphPad Software Inc., CA, USA).

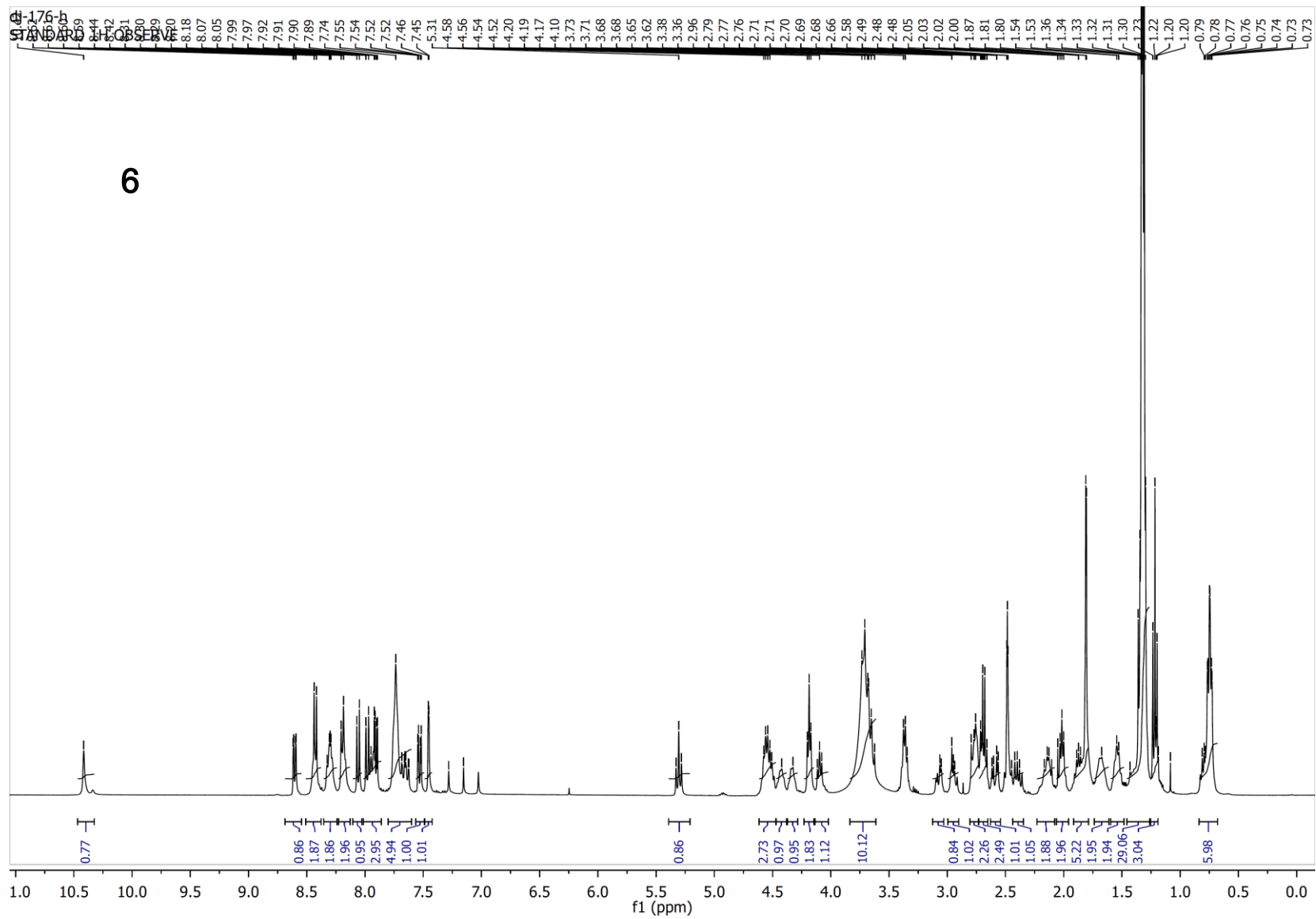
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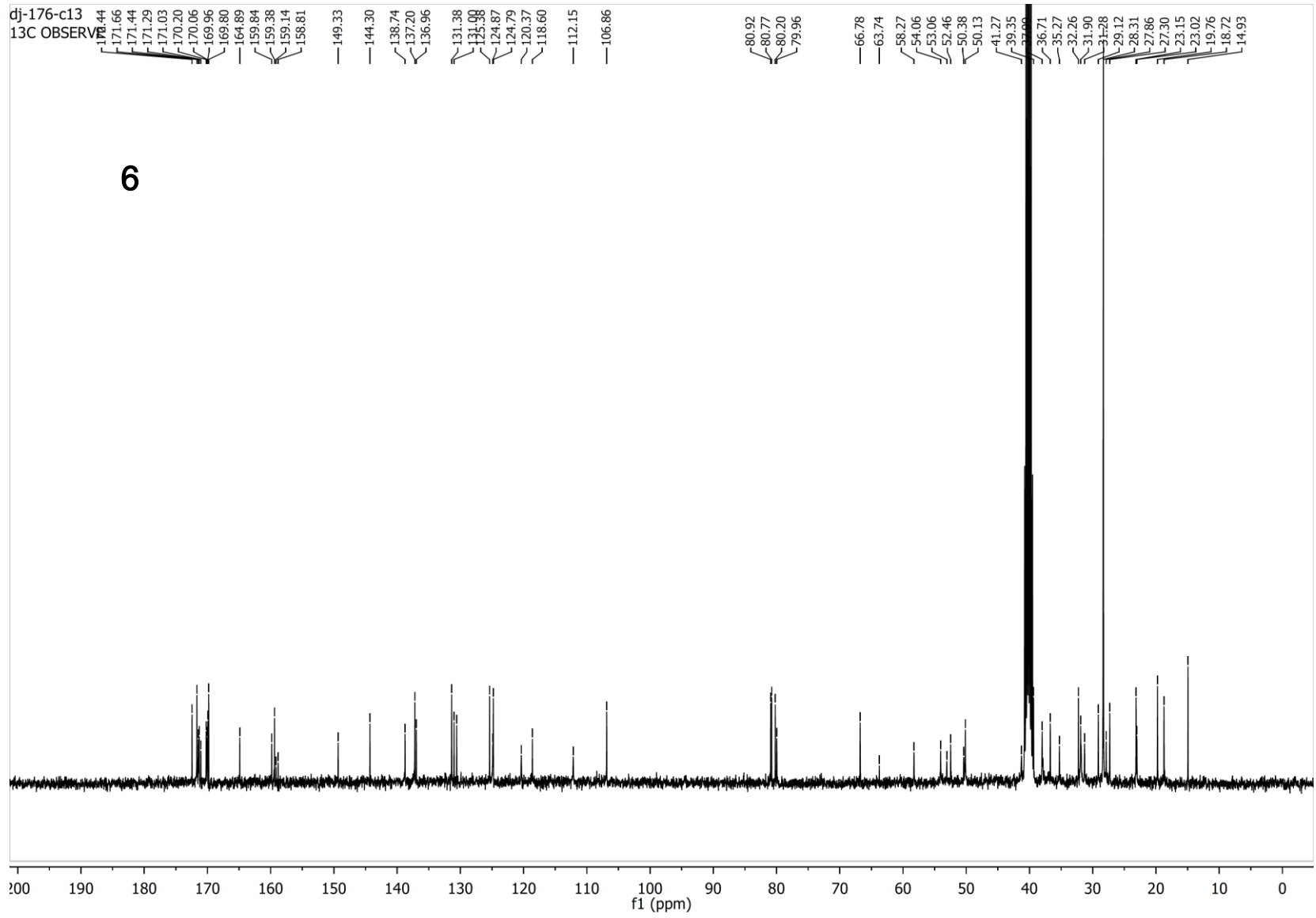
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- [S2] Liang, G., Ren, H. & Rao, J. A biocompatible condensation reaction for controlled assembly of nanostructures in living cells. *Nat. chem.* **2**, 54-60 (2010).

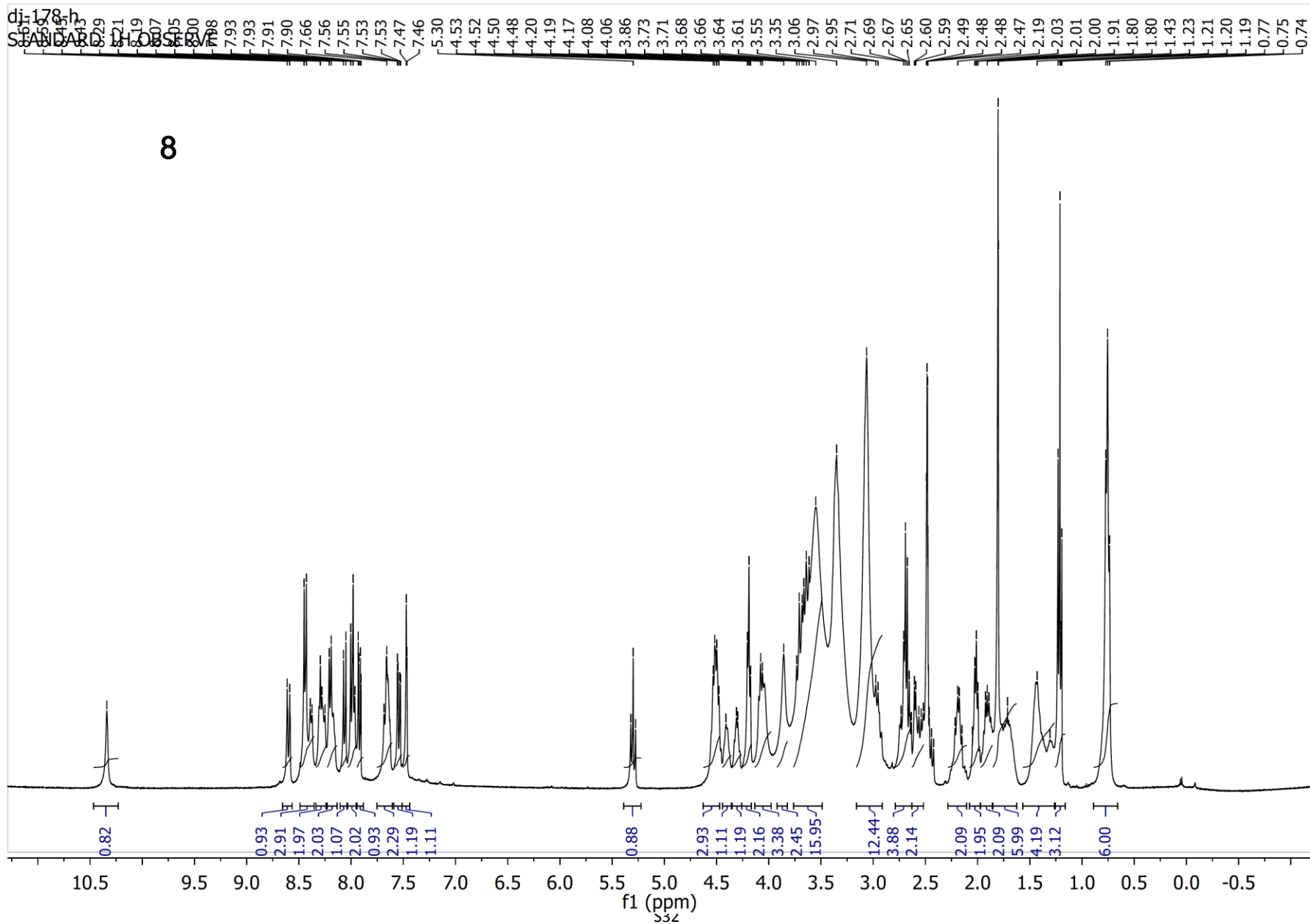
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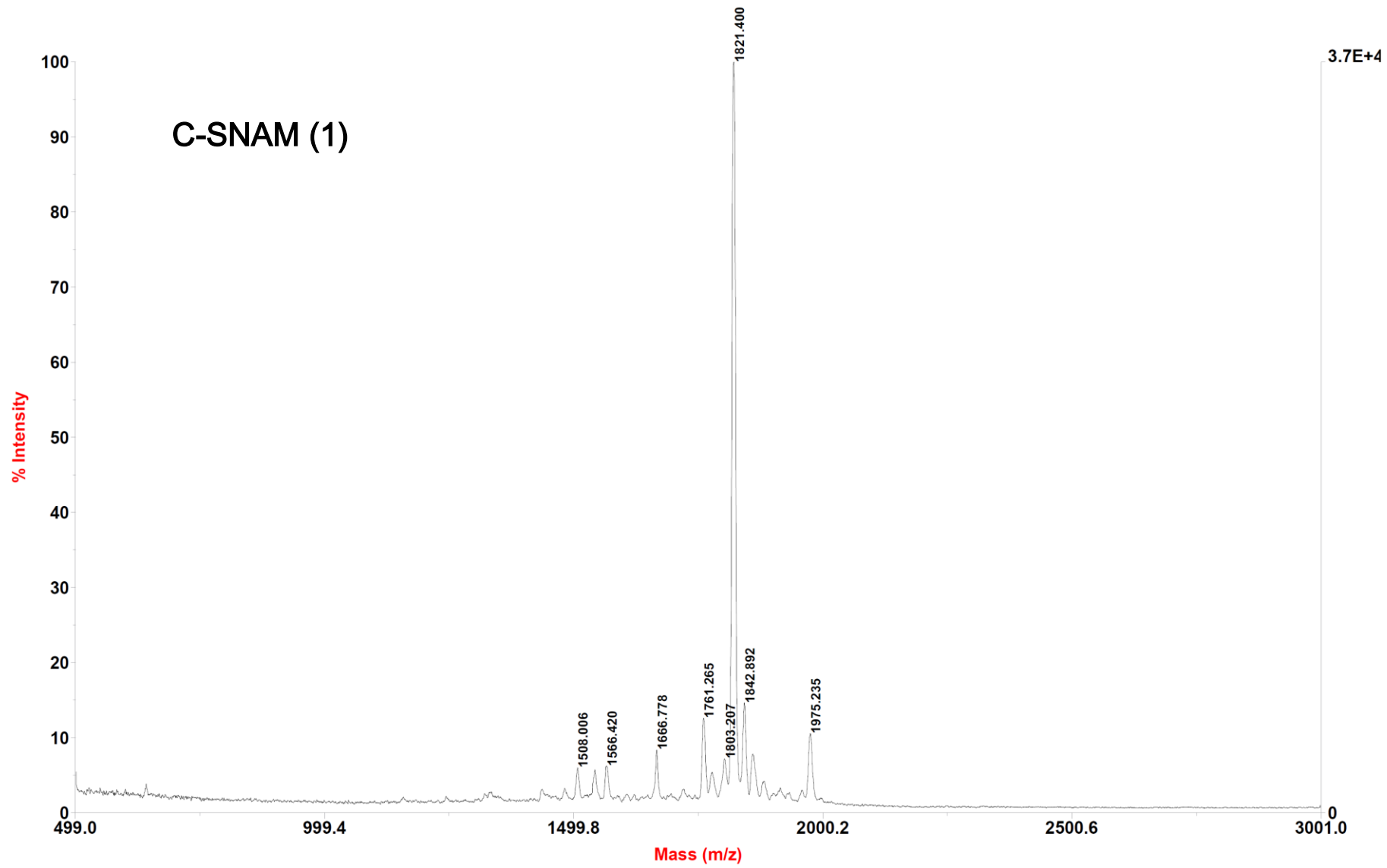
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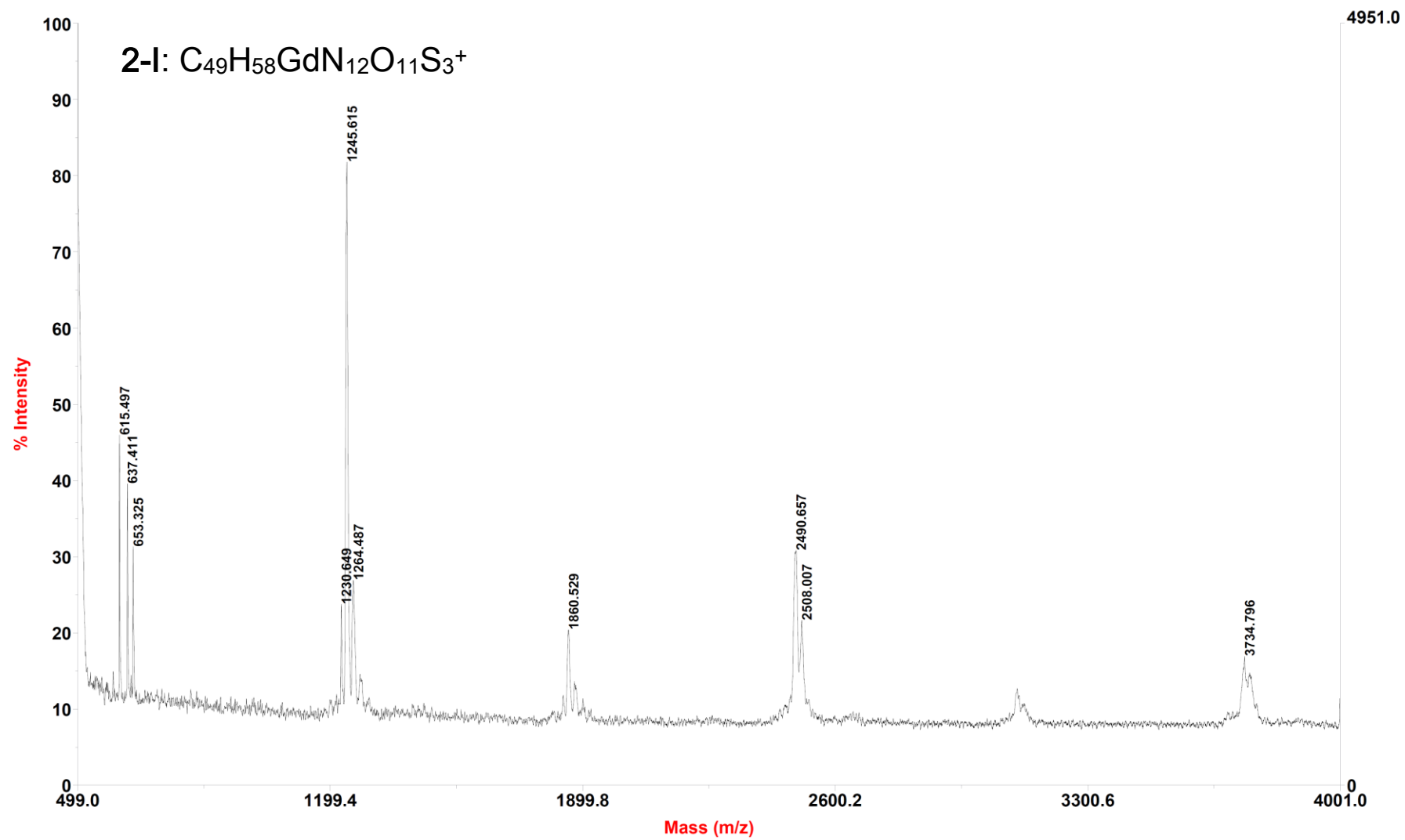


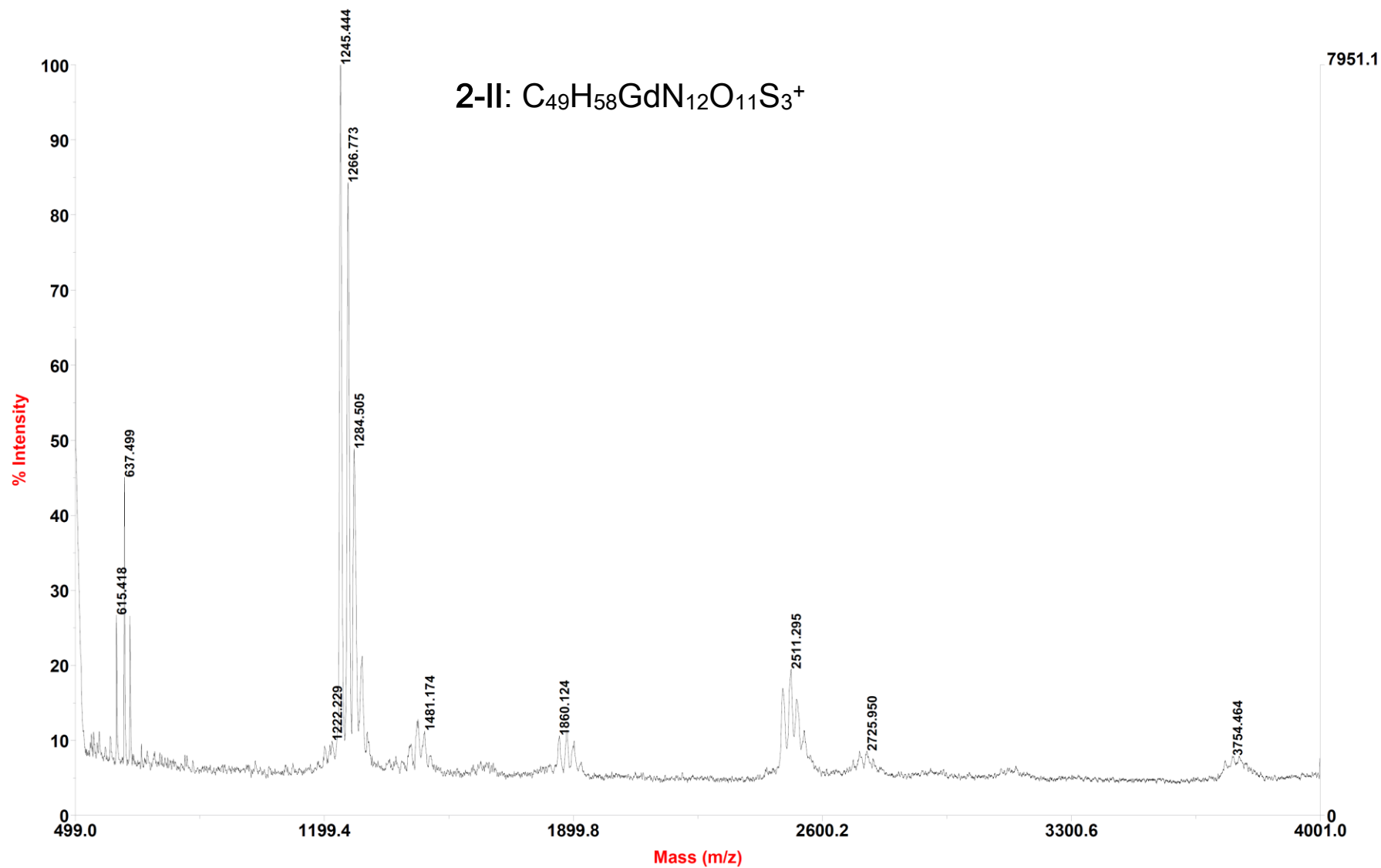












HMRS of 2-I

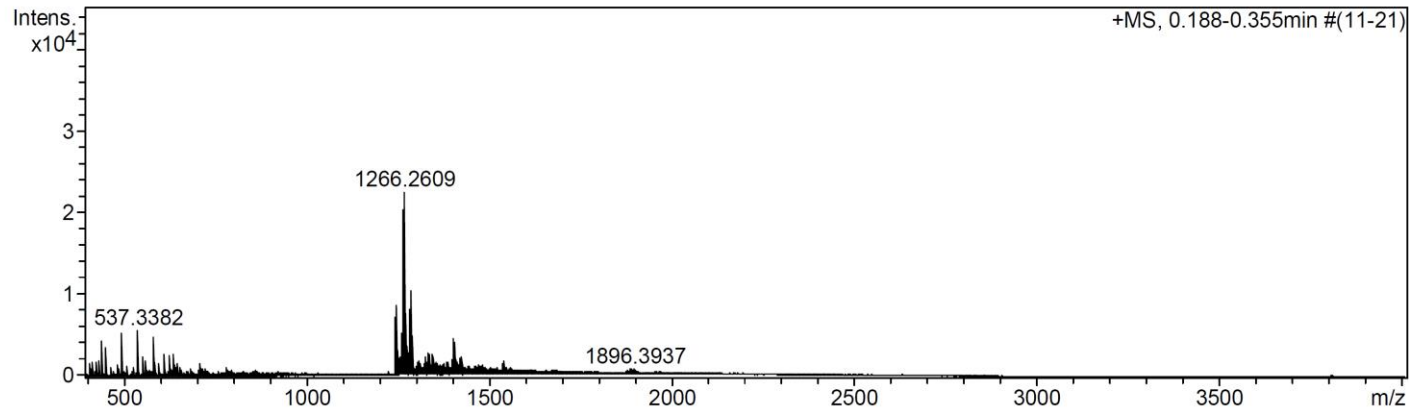
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\HRAM\130712\data\YeD_130712_i343_C-1_03_41_01_2719.d	Acquisition Date	7/12/2013 6:15:46 PM
Method	tune_wide_dirinj_400_4000_smprot.m	Operator	BDAL@DE
Sample Name	YeD_130712_i343_C-1_03	Instrument / Ser#	micrOTOF-Q II 10292
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	2.5 Bar
Focus	Not active	Set Capillary	4200 V	Set Dry Heater	250 °C
Scan Begin	400 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	4000 m/z	Set Collision Cell RF	600.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	z	mSig ma	err [pp m]	Score	err [mD a]	rdb	e ⁻ Conf	N - R ul e
<u>1244.2771</u>	1	<u>C 49 H 58 Gd N 12 O 11 S 3</u>	<u>1244.2751</u>	<u>1+</u>	<u>31.2</u>	<u>-1.6</u>	<u>100.00</u>	<u>-2.0</u>	<u>27.5</u>	<u>even</u>	-
1266.2609	1	C 49 H 57 Gd N 12 Na O 11 S 3	1266.2570		150.6	-3.0	100.00	-3.8	27.5	even	-
	2	C 49 H 64 Gd N 4 O 19 S 3	1266.2569		151.6	-3.1	77.98	-4.0	20.5	even	-

HMRS of 2-I

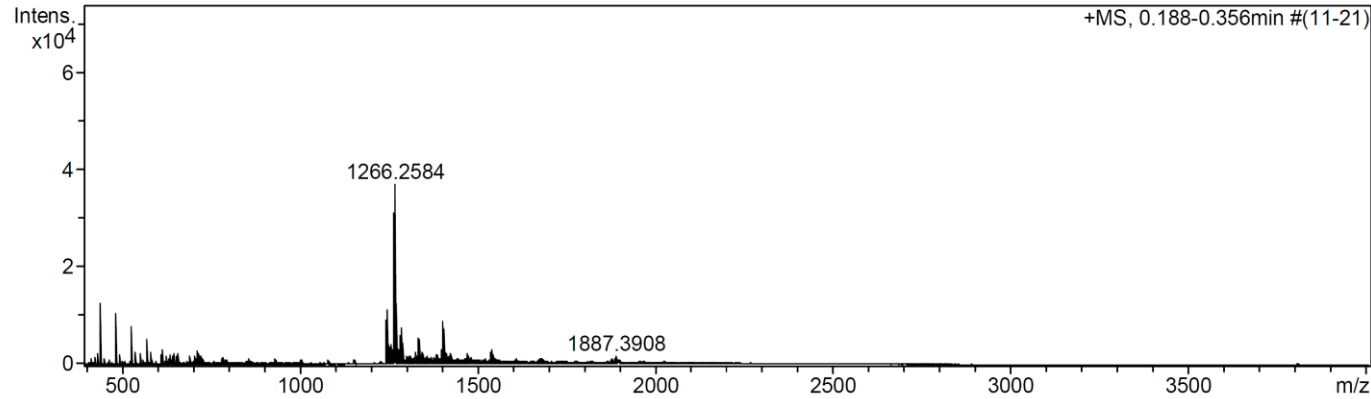
Mass Spectrum SmartFormula Report

Analysis Info

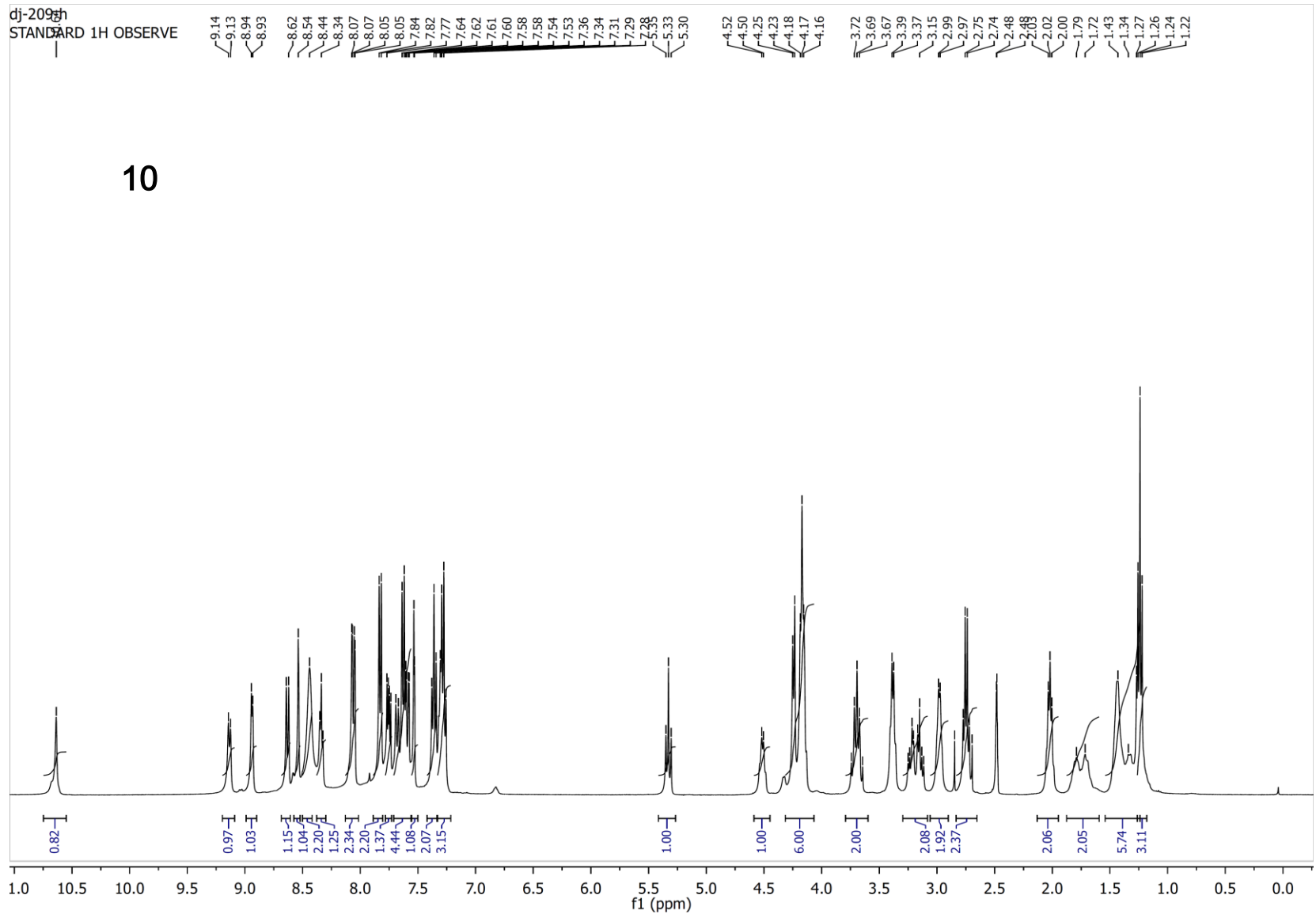
Analysis Name	D:\Data\HRAM\130712\data\YeD_130712_i343_C-2_03_42_01_2721.d	Acquisition Date	7/12/2013 6:21:52 PM
Method	tune_wide_dirinj_400_4000_smprot.m	Operator	BDAL@DE
Sample Name	YeD_130712_i343_C-2_03	Instrument / Ser#	micrOTOF-Q II 10292
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	2.5 Bar
Focus	Not active	Set Capillary	4200 V	Set Dry Heater	250 °C
Scan Begin	400 m/z	Set End Plate Offset	-500 V	Set Dry Gas	8.0 l/min
Scan End	4000 m/z	Set Collision Cell RF	600.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Formula	m/z	z	mSig	err [ppm]	Score	err [mDa]	rdb	e ⁻ Conf	N - Rule
1244.2760	1	C 49 H 51 Gd N 20 Na O 3 S 3	1244.2753	1+	34.4	-0.6	100.00	-0.7	34.5	even	-
	2	C 49 H 58 Gd N 12 O 11 S 3	1244.2751		35.5	-0.7	89.03	-0.9	27.5	even	-
1266.2584	1	C 49 H 57 Gd N 12 Na O 11 S 3	1266.2570		59.2	-1.1	100.00	-1.4	27.5	even	-
	2	C 49 H 64 Gd N 4 O 19 S 3	1266.2569		60.8	-1.2	85.66	-1.5	20.5	even	-



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