Supporting Information

Caspase-3 Responsive Dual-Enhanced $^{1}H/^{19}F$ MRI Bimodal Probe for *In Vivo* Tumor Apoptosis Imaging

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Contents

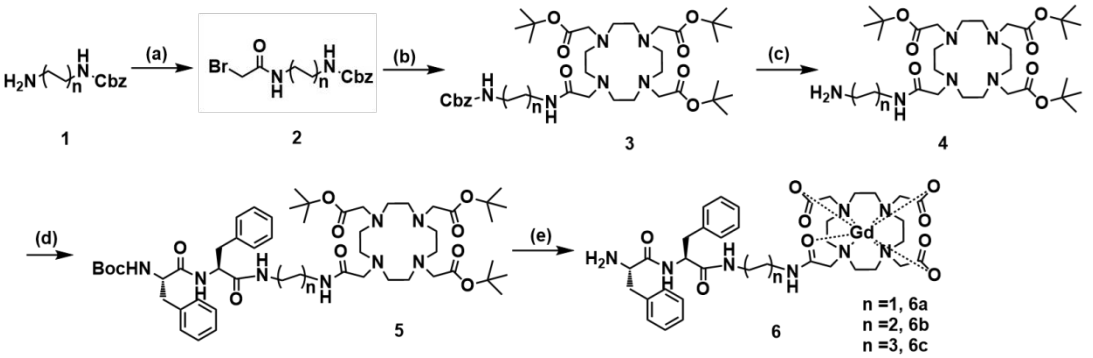
1. Experimental Section

1.1. Materials and instruments

All chemicals were purchased from commercial sources (such as Aldrich, Adamas, Aladdin, and Bide). Commercially available reagents were used without further purification unless noted otherwise. All chemicals were reagent grade or better. Ultrapure water (18.2 MΩ·cm) was purified by a Milli-Q system (Millipore) and used throughout the experiments.

^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker AVANCE III TM 500 MHz spectrometer and referenced to the solvent peak. Data for ^{1}H 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) or DMSO-*d*6 (δ 2.50); 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 nH, and based on the spectral integration values. Mass spectra were obtained on a Liquid Chromatography Triple Quadrupole Mass Spectrometry (LC-MS/MS) in Agilent Technologies. High-performance liquid chromatography (HPLC) was carried out on Shimadzu LC-20A with CH_3CN/H_2O (1‰ CF_3COOH) as the eluents. Longitudinal and transverse relaxation rates were acquired on a 0.5 T NMR relaxometry (Bruker, Germany) and 9.4 T NMR relaxometry (Bruker, Germany). *In vitro* and *in vivo* ^{1}H MRI and ^{19}F MRI were measured on Bruker 400 MHz wide-bore nuclear magnetic resonance spectrometer. The Gd concentration of the sample was measured by ICP-OES (Agilent 5110, USA) or ICP-MS (PQ-MS, Germany). The morphology was measured by transmission electron microscopy (TEM) performed on a Hitachi S-4800 with an electron microscope operating at acceleration voltages of 100 kV using 4% phosphotungstate acid (PTA) for micellar positive stain.

1.2. Synthesis of FF-C_n-Gd



Scheme S1. Synthetic routes of **FF-C_n-Gd**. Reaction conditions: (a) 2-bromoacetyl bromide, $KHCO_3$, DCM, r.t., 4 h, 97.4%-98.4%. (b) Compound **b**, CH_3CN, K_2CO_3, 65 ℃, 8 h, 78.2%-83.7%. (c) Pd/C, H_2, MeOH, r.t., 8 h, 90.0%-95%. (d) Boc-Phe-Phe-OH, DIPEA, DMF, r.t., 2 h, 62.4%-72.3%. (e) TFA/H_2O/TIPS (v: v: v = 95:2.5:2.5), r.t., 1 h, 87.8%; $GdCl_3·6H_2O$, CH_3CN/H_2O (v: v = 1:9), pH = 6.0, r.t., 12 h, 77.4%-92.9%.

Synthesis of compound **2**

To a mixture of benzyl (2-aminoethyl)carbamate (5.0 g, 25.7 mmol); benzyl (4-

aminobutyl)carbamate (5.0 g, 22.5 mmol); benzyl (6-aminohexyl)carbamate (5.0 g, 20.0 mmol) in CH_2Cl_2 (200 mL), $KHCO_3$ (10.8 g, 100.5 mmol), a solution of 2-bromoacetyl bromide (6.5 g, 32.5 mmol) in CH_2Cl_2 (100 mL) was added dropwise at 0 °C, and the reaction was stirred at ambient temperature for 4 h. Then the solution was transferred to the separation funnel, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to afford the products **2a** (6.5 g, 97.4%); **2b** (6.3 g, 96.8%); **2c** (6.3 g, 98.4%) as a white solid.

Compound 2a

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.29 (m, 5H), 5.10 (s, 2H), 3.81 (s, 2H), 3.39 (dd, *J* = 16.0, 10.9 Hz, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.57, 157.27, 136.38, 128.69, 128.38, 128.30, 77.16, 67.13, 41.07, 40.57, 29.01 (Figures S20-S21).

Compound 2b

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.29 (m, 5H), 7.48 – 7.29 (m, 5H), 5.09 (s, 2H), 3.86 (s, 2H), 3.26 (dd, *J* = 40.6, 5.6 Hz, 4H), 1.56 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.58, 156.62, 136.65, 128.67, 128.27, 128.25, 77.16, 66.84, 40.67, 39.92, 29.41, 27.51, 26.61 (Figures S22-S23).

Compound 2c

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 – 7.28 (m, 5H), 5.09 (s, 2H), 3.87 (s, 2H), 3.36 – 3.05 (m, 4H), 1.47 (dd, *J* = 50.2, 43.8 Hz, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.46, 156.59, 136.74, 128.64, 128.22, 77.16, 66.75, 40.90, 40.07, 29.97, 29.47, 29.26, 26.34, 26.22, 0.13 (Figures S24-S25).

Synthesis of compound **3**

To a solution of the compound **b** (3.0 g, 5.1 mmol) and K_2CO_3 (1.9 g, 13.9 mmol) in CH_3CN (150 mL), a solution of the compound **2** (7.0 mmol) in CH_3CN (100 mL) was added, and the reaction was stirred for 8 h at 65 °C. Then the mixture was filtered and the solid part was washed three times with CH_3CN (20 mL). The filtrates were combined and concentrated. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 100:2) to afford product **3** (**3a** 83.7%, **3b** 79.6%, **3c** 78.2%) as a white solid.

Compound 3a

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.35 (s, 1H), 7.30 (d, *J* = 7.2 Hz, 2H), 7.26 – 7.22 (m, 2H), 7.19 (t, *J* = 7.1 Hz, 1H), 6.43 (s, 1H), 5.01 (s, 2H), 3.34 (d, *J* = 31.5 Hz, 10H), 2.64 – 1.88 (m, 16H), 1.38 (d, *J* = 11.7 Hz, 27H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.35, 172.23, 156.80, 137.12, 128.28, 127.78, 127.68, 127.60, 81.85, 77.16, 66.03, 56.05, 55.65, 53.50, 50.34, 40.77, 39.48, 27.95, 27.90 (Figures S26-S27).

Compound 3b

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32 (s, 5H), 5.05 (s, 2H), 3.70 – 3.09 (m, 10H), 3.06 – 2.52 (m, 8H), 2.15 (ddd, *J* = 88.5, 21.3, 10.1 Hz, 8H), 1.52 (d, *J* = 33.9 Hz, 6H), 1.42 (d, *J* = 8.3 Hz, 25H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.45, 171.67, 156.68, 137.03, 128.54, 128.50, 128.05, 127.99, 127.92, 82.01, 77.16, 66.33, 56.28, 55.83, 40.77, 38.70, 28.08, 28.03, 27.15 (Figures S28-S29).

Compound 3c

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.15 (s, 1H), 7.32 – 7.27 (m, 4H), 7.25 – 7.19 (m, 1H), 5.01 (s, 2H), 3.40 – 3.22 (m, 4H), 3.12 (tt, *J* = 12.8, 6.5 Hz, 6H), 3.01 – 2.74 (m, 6H), 2.62 – 2.04 (m, 12H), 1.53 – 1.45 (m, 4H), 1.38 (d, *J* = 6.8 Hz, 27H), 1.25 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.38, 171.44, 156.45, 136.77, 128.41, 127.88, 81.77, 81.68, 77.41, 77.16, 77.16, 76.91, 66.30, 56.20, 55.72, 55.55, 40.83, 39.02, 29.69, 29.22, 28.14, 28.10, 28.01, 27.97, 27.90, 26.50, 26.26 (Figures S30-S31).

Synthesis of compound **4**

Compound **3** was stirred over Pd/C (10 % by wt.) in MeOH under 1.0 atm H_2 (g). After 8 h, the mixture was filtered to remove Pd/C and the solid part was washed three times with CH_2Cl_2 (10 mL). The filtrate was combined and concentrated to afford product **4** as a white solid.

Synthesis of compound **5**

Boc-Phe-Phe-OH (0.22 mmol), HATU (0.2 mmol) and DIPEA (0.4 mmol) were added to compound **4** (0.2 mmol). After 2 h reaction, the reaction liquid was combined and concentrated. The residue was purified by chromatography on a silica gel column (CH_2Cl_2/MeOH = 100:2) to afford product **5** as a white solid.

Compound 5a

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.25 – 7.11 (m, 10H), 3.65 (s, 2H), 3.13 – 1.96 (m, 32H), 1.48 – 1.38 (m, 27H), 1.27 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 7.27, 7.26, 7.24, 7.23, 7.21, 7.20, 7.19, 7.17, 7.16, 7.15, 7.14, 7.13, 3.65, 3.10, 3.07, 3.07, 3.06, 3.05, 3.04, 3.04, 3.03, 3.03, 3.02, 3.01, 3.00, 2.98, 2.95, 2.93, 2.91, 2.85, 2.78, 2.71, 2.65, 2.64, 2.54, 2.31, 2.29, 2.13, 2.02, 1.44, 1.43, 1.42, 1.27 (Figures S32-S33).

Compound 5b

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.19 (dd, *J* = 10.2, 3.9 Hz, 6H), 7.10 (d, *J* = 9.2 Hz, 2H), 7.05 (d, *J* = 7.0 Hz, 2H), 6.48 (d, *J* = 4.7 Hz, 2H), 3.62 (s, 2H), 3.13 (d, *J* = 4.4 Hz, 4H), 3.06 – 2.93 (m, 6H), 2.91 (d, *J* = 1.7 Hz, 6H), 2.83 (d, *J* = 1.8 Hz, 6H), 2.76 (s, 2H), 2.32 – 1.90 (m, 6H), 1.41 (d, *J* = 7.4 Hz, 27H), 1.34 (d, *J* = 6.6 Hz, 6H), 1.29 (s, 3H), 1.25 (s, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.56, 171.40, 162.56, 136.67, 136.60, 135.73, 135.67, 129.35, 129.33, 129.21, 129.17, 128.67, 128.57, 128.50, 127.09, 127.05, 126.87, 126.75, 81.81, 81.74, 80.36, 77.16, 55.97, 55.69, 53.51, 53.30, 53.13, 52.24, 38.87, 38.59, 36.48, 31.39, 28.21, 28.09, 27.94, 27.91 (Figures S36-S37).

Compound 5c

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 – 7.27 (m, 1H), 7.25 – 6.94 (m, 9H), 6.53 – 6.40 (m, 2H), 3.12 (d, *J* = 26.2 Hz, 6H), 3.00 (dd, *J* = 13.9, 5.4 Hz, 2H), 2.95 – 2.90 (m, 2H), 2.85 (s, 2H), 2.78 (s, 4H), 2.56 – 1.77 (m, 12H), 1.48 – 1.35 (m, 32H), 1.32 (s, 4H), 1.28 (d, *J* = 11.6 Hz, 6H), 1.25 – 1.11 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.63, 171.47, 170.46, 162.65, 136.76, 136.49, 136.28, 129.41, 129.37, 129.33, 128.84, 128.71, 128.67, 127.07, 126.99, 81.89, 81.82, 80.67, 77.16, 56.79, 56.53, 56.02, 55.80, 55.65, 54.42, 53.94, 53.56, 39.35, 39.17, 38.68, 29.04, 29.01, 28.28, 28.16, 28.04, 28.00, 26.22, 26.13 (Figures S40-S41).

Synthesis of compound **6**

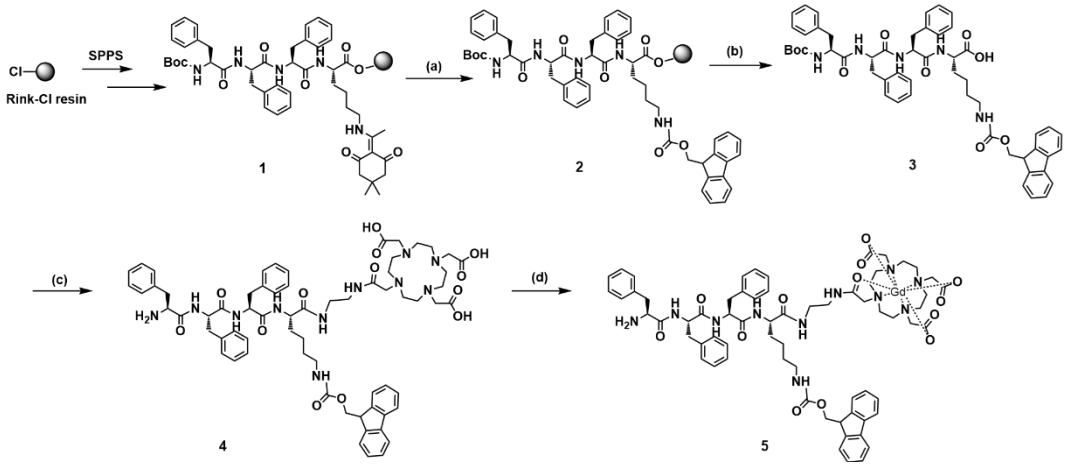
After that, a solution of compound **5** (0.1 mmol) in H_2O was carefully added to a solution of 0.1 M NaOH to adjust pH value to ~6.0. Then, a solution of $GdCl_3 \cdot 6H_2O$ (40.9 mg, 0.11 mmol) in 1 mL water was added. After stirring at r.t. for 10 min, the pH value of the reaction solution was further adjusted to ~6.0 using 0.1 M NaOH, and the reaction mixture was stirred at r.t. overnight. After the reaction, the reaction mixture was purified by HPLC to give compound **6** as a white solid.

Compound 6a HRMS (ESI) Calcd for $C_{36}H_{50}GdN_8O_9$, $[M + H]^+$: 896.2947, found: 896.2958 (Figure S34).

Compound 6b HRMS (ESI) Calcd for $C_{38}H_{54}GdN_8O_9$, $[M + H]^+$: 924.3255, found: 924.3278 (Figure S38).

Compound 6c HRMS (ESI) Calcd for $C_{40}H_{54}GdN_8O_9$, $[M + H]^+$: 952.3568, found: 952.3585 (Figure S42).

1.3. Synthesis of FFFK(Fmoc)-C_2-Gd



Scheme S2. Synthetic routes of **FFFK(Fmoc)-C_2-Gd (5)**. Reaction conditions: (a) 2% $NH_2NH_2 \cdot H_2O$, 9-fluorenylmethyl chloroformate, DIPEA, r.t., 2 h, 81.6%. (b) TFA/DCM (v: v = 1:99), r.t., 15 min, 55.4%. (c) Compound **d**, HATU, DIPEA, r.t., 2 h, 57.2%; TFA/H_2O/TIPS (v: v: v = 95:2.5:2.5), r.t., 1 h, 87.8%. (d) $GdCl_3 \cdot 6H_2O$, CH_3CN/H_2O (v: v = 1:9), pH = 6.0, r.t., 12 h, 92.9%.

Synthesis of compound **3**

SPPS (2.0 g, 2 mmol, 2-chlorotrityl chloride resin) was used to prepare the peptide Boc-Phe-Phe-Phe-Lys (Dde)-OH-Resin (**1**). Then, the Dde protecting group of compound **1** was cleaved with 2% hydrazine in DMF (5 mL) for 10 min and 9-fluorenylmethyl chloroformate was added to protect the amino group to obtain the Boc-Phe-Phe-Phe-Lys (Fmoc)-OH-Resin (**2**). After that, the peptide was released from the resin by 1% TFA (TFA/DCM v: v = 1:99) for 15 min. Cold diethyl ether (Et_2O, 20 mL) was then added, and the resulting precipitate was centrifuged to give the compound **3** as a light yellow solid.

^{1}H NMR (500 MHz, DMSO-*d*6) δ 12.67 (s, 1H), 8.24 (dd, *J* = 17.2, 7.8 Hz, 2H), 7.90 (t, *J* = 19.7 Hz, 3H), 7.78 – 7.06 (m, 22H), 6.86 (d, *J* = 8.7 Hz, 1H), 4.80 – 4.49 (m, 2H), 4.47 – 3.96 (m, 5H), 3.16 – 2.94 (m, 4H), 2.82 (td, *J* = 13.9, 7.1 Hz, 3H), 2.62 (dd, *J* = 13.4, 10.9 Hz, 1H), 1.83 – 1.54 (m, 2H), 1.49 – 1.00 (m, 13H). ^{13}C NMR (126 MHz, DMSO-*d*6) δ 173.49, 171.27, 170.95, 170.70,

156.12, 155.04, 143.96, 140.77, 138.14, 137.57, 137.53, 129.38, 129.24, 129.13, 128.04, 127.94, 127.60, 127.06, 126.26, 126.19, 126.09, 125.16, 120.11, 78.07, 65.23, 55.83, 53.56, 53.42, 52.00, 46.82, 39.52, 37.80, 37.62, 37.57, 35.78, 30.87, 30.78, 29.09, 28.10, 27.70, 22.73 (Figures S44-S45).

HRMS (ESI) Calcd for $C_{48}H_{52}N_5O_7$, $[M + H]^+$: 810.3867, found: 810.4500 (Figure S46).

Synthesis of compound **4**

Next, compound **d** (135.0 mg, 0.22 mmol), HATU (76.4 mg, 0.2 mmol), and DIPEA (67.9 μL, 0.4 mmol) were added into compound **3** (181.8 mg, 0.2 mmol). After 2 h reaction, the crude product was directly added into a solution of TIPSH (0.02 mmol) in TFA/DCM/CH_3CN (v: v: v = 95/2.5/2.5, 10 mL) stirred at r.t. for 1 h to remove the OtBu group completely. The solvent was then removed. Cold Et_2O (20 mL) was added and the precipitate was dried under vacuum and purified by HPLC to give intermediate compound **4** as a white solid.

^{1}H NMR (500 MHz, DMSO-*d*6) δ 8.74 (d, *J* = 8.2 Hz, 1H), 8.52 (s, 1H), 8.41 (d, *J* = 7.8 Hz, 1H), 8.17 (d, *J* = 6.7 Hz, 1H), 8.04 (s, 2H), 7.94 (s, 1H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.67 (d, *J* = 7.4 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.26 (dtt, *J* = 23.1, 13.5, 6.7 Hz, 18H), 4.64 (ddd, *J* = 20.8, 13.0, 8.5 Hz, 3H), 4.29 (d, *J* = 6.9 Hz, 2H), 4.20 (t, *J* = 6.5 Hz, 2H), 4.02 (d, *J* = 21.6 Hz, 3H), 3.84 (s, 2H), 3.64 (s, 3H), 3.35 (s, 6H), 3.25 – 3.19 (m, 2H), 3.17 – 2.93 (m, 15H), 2.83 (ddd, *J* = 23.1, 13.9, 8.6 Hz, 4H), 1.65 (d, *J* = 7.3 Hz, 1H), 1.59 – 1.48 (m, 1H), 1.41 (d, *J* = 6.6 Hz, 2H), 1.27 (dd, *J* = 19.4, 10.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-*d*6) δ 174.09, 174.06, 171.86, 171.86, 171.71, 171.69, 171.50, 171.35, 170.90, 170.87, 170.85, 170.76, 170.73, 170.66, 163.70, 163.68, 158.25, 158.00, 136.08, 136.07, 130.89, 130.63, 130.36, 130.10, 128.39, 126.41, 125.12, 124.24, 122.07, 120.47, 119.90, 118.10, 115.73, 113.36, 58.37, 57.93, 54.67, 53.91, 52.73, 52.35, 52.15, 50.92, 50.84, 50.45, 49.86, 49.72, 48.61, 39.52, 35.92, 35.74, 30.42, 30.21, 30.16, 26.78, 26.64, 19.08, 19.01, 18.22, 17.98 (Figures S47-S48).

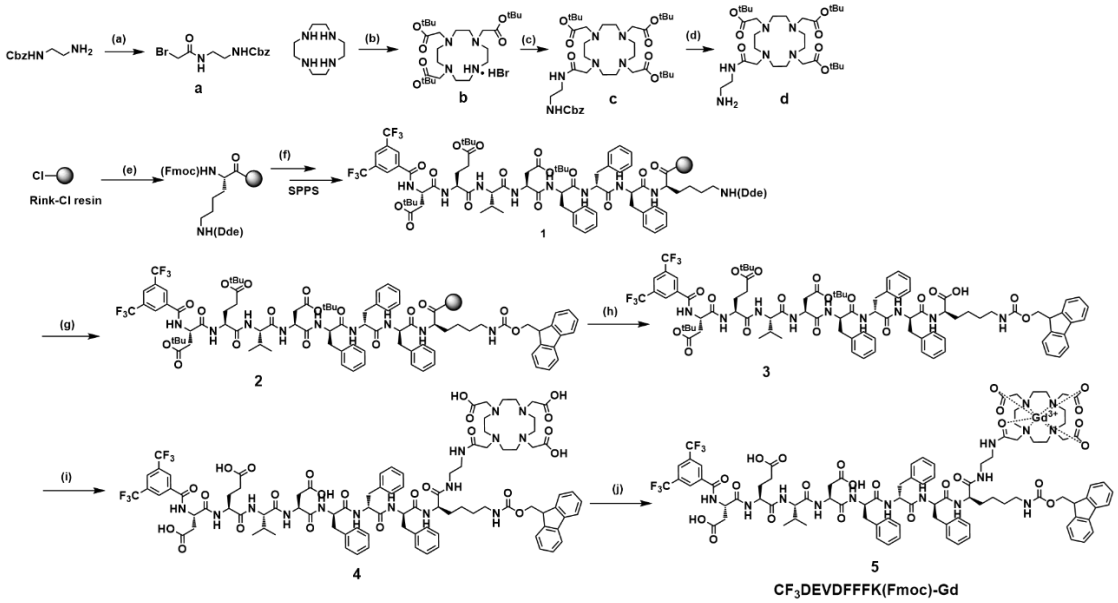
HRMS (ESI) Calcd for $C_{66}H_{84}N_{11}O_{13}$, $[M + H]^+$: 1238.6250, found: 1238.6254 (Figure S49).

Synthesis of compound **FFFK(Fmoc)-C_2-Gd** (**5)**

After that, a solution of compound **4** (123.7 mg, 0.1 mmol) in CH_3CN/H_2O (v: v = 1:9) was carefully added to a solution of 0.1 M NaOH to adjust pH value to ~6.0. Then, a solution of $GdCl_3 \cdot 6H_2O$ (40.9 mg, 0.11 mmol) in 1 mL water was added. After stirring at r.t. for 10 min, the pH value of the reaction solution was further adjusted to ~6.0 using 0.1 M NaOH, and the reaction mixture was stirred at r.t. overnight. After the reaction, the reaction mixture was purified by HPLC to give **FFFK(Fmoc)-C_2-Gd** as a white solid (Yield: 92.9%, Purity: 98.83%)(Figure S50).

HRMS (ESI) Calcd for $C_{66}H_{81}GdN_{11}O_{13}$, $[M + H]^+$: 1393.5256, found: 1393.5260 (Figure S51).

1.4. Synthesis of CF_3DEVDFFFK(Fmoc)-Gd



Scheme S3. Synthetic routes of **CF_3DEVDFFFK(Fmoc)-Gd**. Reaction conditions: (a) 2-bromoacetyl bromide, $KHCO_3$, DCM, r.t., 4 h, 97.4%. (b) tert-butyl 2-bromoacetate, CH_3COONa, DMA, r.t., 8 h, 65.5%. (c) benzyl (2-(2-bromoacetamido) ethyl) carbamate, CH_3CN, K_2CO_3, 65 ℃, 8 h, 83.7%. (d) Pd/C, H_2, MeOH, r.t., 8 h, 95.0%. (e) Fmoc-Lys (Dde)-OH, DIPEA, DMF, r.t., 2 h, 89.7%. (f) Fmoc-Phe-OH, Fmoc-Asp (OtBu)-OH, Fmoc-Glu (OtBu), Fmoc-Val-OH, 3,5-bis(trifluoromethyl) benzoic acid, HATU, DIPEA, DMF, r.t., 2 h. (g) 2% $NH_2NH_2 \cdot H_2O$, 9-fluorenylmethyl chloroformate, DIPEA, r.t., 2 h. (h) TFA/DCM (v: v = 1:99), r.t., 15 min, 29.3%. (i) Compound **d**, HATU, DIPEA, r.t., 2 h, 51.4%; TFA/H_2O/TIPS (v: v: v = 95:2.5:2.5), r.t., 1 h, 97.8%. (j) $GdCl_3 \cdot 6H_2O$, CH_3CN/H_2O (v: v = 1:9), pH = 6.0, r.t., 12 h, 92.4%.

Synthesis of **benzyl (2-(2-bromoacetamido) ethyl) carbamate (a)**

To a mixture of benzyl (2-aminoethyl) carbamate (5.0 g, 21.7 mmol) in CH_2Cl_2 (200 mL), $KHCO_3$ (10.8 g, 100.5 mmol), a solution of 2-bromoacetyl bromide (6.5 g, 32.5 mmol) in CH_2Cl_2 (100 mL) was added dropwise at 0 °C, and the reaction was stirred at ambient temperature for 4 h. Then the solution was transferred to the separation funnel, and the aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to afford the product **a** (6.5 g, 97.4%) as a white solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.29 (m, 5H), 5.10 (s, 2H), 3.81 (s, 2H), 3.39 (dd, J = 16.0, 10.9 Hz, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.57, 157.27, 136.38, 128.69, 128.38, 128.30, 77.16, 67.13, 41.07, 40.57, 29.01 (Figures S52-S53).

Synthesis of **tri-tert-butyl 2,2',2''-(1,4,7,10-tetraazacyclododecane-1,4,7-triyl) triacetate (b)**

To a mixture of 1,4,7,10-tetraazacyclododecane (2.0 g, 11.6 mmol) and CH_3COONa (2.8 g, 34.8 mmol) in DMA (100 mL), a solution of t-butyl 2-bromoacetate (6.8 g, 34.8 mmol) in DMA (100 mL) was added dropwise at 0 °C, and the reaction was stirred at ambient temperature for 8 h. Then the mixture was filtered and the solid part was washed three times with DMA (10 mL). The filtrates were combined and concentrated to afford the product **b** as a white solid (4.5 g, 65.5%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.26 (d, *J* = 44.1 Hz, 6H), 2.84 (t, *J* = 16.9 Hz, 14H), 2.00 (d, *J* = 4.6 Hz, 3H), 1.39 (d, *J* = 3.6 Hz, 27H) (Figure S54).
HRMS (ESI) Calcd for $C_{26}H_{50}N_4O_6$, $[M + H]^+$: 515.3809, found: 515.3811 (Figure S55).

Synthesis of **tri-tert-butyl 2,2',2''-(10-(2-((2-(((benzyloxy)carbonyl) amino) ethyl) amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl) triacetate (c)**
To a solution of the compound **b** (3.0 g, 5.1 mmol) and K_2CO_3 (1.9 g, 13.9 mmol) in CH_3CN (150 mL), a solution of the compound **a** (2.2 g, 7.0 mmol) in CH_3CN (100 mL) was added, and the reaction was stirred for 8 h at 65 °C. Then the mixture was filtered and the solid part was washed three times with CH_3CN (20 mL). The filtrates were combined and concentrated. The residue was purified by chromatography on silica gel column (CH_2Cl_2/MeOH = 100:2) to afford product **c** (3.2 g, 83.7%) as a white solid.
^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31 – 7.30 (d, *J* = 7.3 Hz, 2H), 7.27 – 7.24 (m, 2H), 7.21 – 7.18 (t, *J* = 7.2 Hz, 1H), 5.02 (s, 2H), 3.33 (s, 9H), 2.95 – 2.03 (m, 19H), 1.40 – 1.37 (d, *J* = 12.3 Hz, 27H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 172.29, 156.80, 137.13, 128.28, 127.78, 127.60, 81.85, 66.03, 56.05, 55.65, 53.50, 50.34, 40.77, 39.48, 27.98 (Figures S56-S57).
HRMS (ESI) Calcd for $C_{38}H_{65}N_6O_9$, $[M + H]^+$: 749.4813, found: 749.4815 (Figure S58).

Synthesis of **tri-tert-butyl 2,2',2''- (10- (2- ((2-aminoethyl) amino)-2-oxoethyl)-1, 4, 7, 10-tetraazacyclododecane-1, 4, 7- triyl) triacetate (d)**
Compound **c** (1.0 g, 1.3 mmol) was stirred over Pd/C (100.0 mg, 10 % by wt.) in MeOH under 1.0 atm H_2 (g). After 8 h, the mixture was filtered to remove Pd/C, and the solid part was washed three times with CH_2Cl_2 (10 mL). The filtrate was combined and concentrated to afford product **d** (0.72 g, 89.7%) as a white solid.
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.45 (dd, *J* = 15.8, 10.4 Hz, 1H), 4.02 (t, *J* = 11.2 Hz, 2H), 3.40 – 3.20 (m, 8H), 2.92 – 2.70 (m, 8H), 2.60 (s, 5H), 2.34 – 2.07 (m, 5H), 1.38 (s, 27H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 173.77, 172.43, 172.10, 81.85, 62.09, 56.07, 55.65, 50.11 (s), 42.49, 41.71, 41.46, 27.94 (Figures S59-S60).
HRMS (ESI) Calcd for $C_{30}H_{59}N_6O_7$, $[M + H]^+$: 615.4445, found: 615.4449 (Figure S61).

Synthesis of **CF_3-Asp (OtBu)-Glu (OtBu)-Val-Asp (OtBu)-Phe-Phe-Phe-Lys (Fmoc)-OH (3)**
Compound **3** was synthesized by standard solid-phase peptide synthesis (SPPS) using 2-chlorotrityl chloride resin (2.0 g, 2 mmol) and the corresponding Fmoc-protected amino acids with side chains properly protected. The first amino acid Fmoc-Lys (Dde)-OH was loaded onto the resin at about 1.0 mmol/g of resin. After loading the first amino acid to the resin, the capping regent (DCM: MeOH: DIPEA = 17:2:1) was used to ensure all the active sites of the resin were protected. The solution of 20% piperidine in DMF was used to remove the Fmoc group and the next Fmoc-protected amino acid was coupled to the free amino group using HATU as the coupling reagent. The growth of the peptide chain followed the established Fmoc SPPS protocol. The peptide CF_3-Asp (OtBu)-Glu (OtBu)-Val-Asp (OtBu)-Phe-Phe-Phe-Lys (Dde)-OH-Resin (**1**) was prepared. After that, the side chain protect group Dde was hydrolyzed by 2% hydrazine in DMF (5 mL) for 10 min and washed with DMF/DCM twice. Then the 9-fluorenylmethyl chloroformate (1.5 g, 6 mmol), DIPEA (678 μL, 4 mmol) were added to the resin. After 30 min reaction, the compound **2** was prepared. Fluorenylmethyl chloroformate was used to protect the side chain amino group. The resin was

washed by the DMF/DCM and released by TFA/DCM (v: v = 1:99). The peptide CF_3-Asp (OtBu)-Glu (OtBu)-Val-Asp (OtBu)-Phe-Phe-Phe-Lys (Fmoc)-OH (**3**) was obtained (984.0 mg, yield: 29.3%) after cleaving the peptide from the resin and further through HPLC purification using water-acetonitrile added with 0.1% TFA as the eluent from 2:8 to 1:9.

^{1}H NMR (500 MHz, DMSO-*d*6) δ 12.63 (s, 1H), 9.27 (d, *J* = 7.8 Hz, 1H), 8.50 (s, 2H), 8.33 (d, *J* = 17.2 Hz, 1H), 8.21 (dd, *J* = 13.1, 7.8 Hz, 3H), 8.13 (d, *J* = 8.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.69 (dd, *J* = 14.4, 8.0 Hz, 4H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.36 – 7.07 (m, 18H), 4.85 (td, *J* = 8.5, 5.5 Hz, 1H), 4.66 – 4.48 (m, 3H), 4.43 (dd, *J* = 12.5, 7.9 Hz, 1H), 4.35 – 4.24 (m, 3H), 4.18 (dd, *J* = 14.7, 8.3 Hz, 3H), 3.06 (dt, *J* = 19.5, 9.9 Hz, 1H), 3.02 – 2.94 (m, 3H), 2.91 – 2.59 (m, 6H), 2.54 (dd, *J* = 8.6, 6.0 Hz, 1H), 2.41 – 2.08 (m, 3H), 1.94 – 1.81 (m, 2H), 1.79 – 1.66 (m, 2H), 1.66 – 1.53 (m, 1H), 1.46 – 1.38 (m, 2H), 1.37 – 1.13 (m, 29H), 0.79 – 0.66 (m, 6H).

^{13}C NMR (126 MHz, DMSO-*d*6) δ 173.43, 171.75, 170.90, 170.73, 170.65, 170.50, 170.21, 170.04, 169.83, 169.26, 169.09, 163.46, 156.09, 143.94, 140.74, 137.58, 137.56, 137.31, 136.19, 130.88, 130.62, 130.35, 130.09, 129.24, 129.19, 129.13, 128.30, 128.03, 127.95, 127.89, 127.58, 127.04, 126.36, 126.23, 126.15, 126.08, 125.14, 124.19, 122.02, 120.10, 119.85, 80.20, 80.13, 79.49, 65.19, 57.07, 53.58, 52.00, 51.97, 50.53, 49.47, 46.78, 39.52, 37.58, 37.43, 37.04, 31.28, 30.95, 30.82, 29.06, 27.61, 27.59, 27.09, 22.69, 19.19, 17.68, 18.79 (Figures S62-S63).

HRMS (ESI) Calcd for $C_{75}H_{80}F_6N_9O_{18}$, $[M + H]^+$:1508.5526, found:1508.4500 (Figure S64).

Synthesis of **CF_3-Asp-Glu-Val-Asp-Phe-Phe-Phe-Lys (Fmoc)-DO3A (4)**

Compound 3 (335.0 mg, 0.2 mmol), DIPEA (67.9 μL, 0.4 mmol) and HATU (76.0 mg, 0.2 mmol) were dissolved in dry DMF (2 mL) and stirred at r.t. for 5 min. Then compound **d** (122.8 mg, 0.2 mmol) was dropped into the reaction and stirred at r.t. for 2 h. The DMF was removed under vacuum and the residue was directly dissolved in a solution of Triisopropylsilane (TIPSH, 0.02 mmol) in TFA/DCM/CH_3CN (v: v: v = 95/2.5/2.5, 10 mL) and the mixture was kept stirring at room temperature for 3 h to remove the OtBu groups. The solvent was removed under reduced pressure. Cold diethyl ether (Et_2O, 20 mL) was then added and the resulting precipitate was centrifuged to give the intermediate as a white solid. The crude product was then purified by HPLC to afford compound **4** as a white powder.

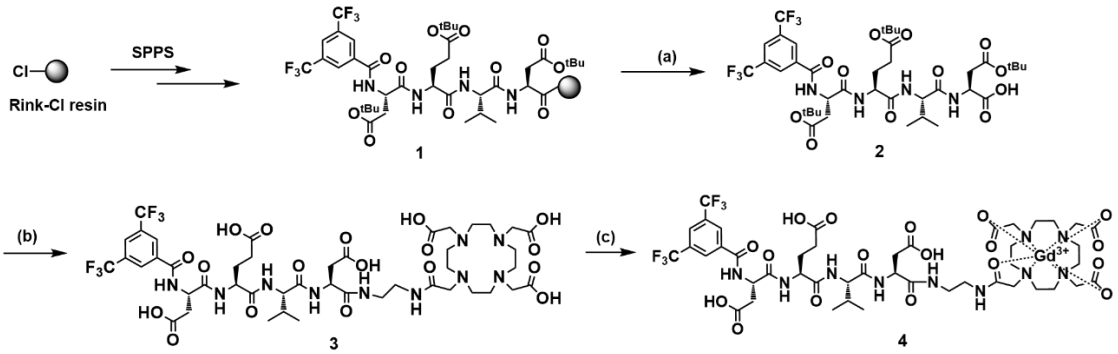
^{1}H NMR (500 MHz, DMSO-*d*6) δ 9.23 (d, *J* = 7.2 Hz, 1H), 8.52 (s, 2H), 8.42 (s, 1H), 8.33 (s, 1H), 8.27 (d, *J* = 7.7 Hz, 1H), 8.19 (d, *J* = 7.4 Hz, 1H), 8.09 – 7.93 (m, 3H), 7.88 (d, *J* = 7.5 Hz, 2H), 7.84 – 7.70 (m, 2H), 7.67 (d, *J* = 7.4 Hz, 2H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.34 – 7.07 (m, 18H), 4.82 (ddd, *J* = 9.4, 7.5, 4.7 Hz, 1H), 4.56 (ddd, *J* = 21.1, 13.5, 7.8 Hz, 3H), 4.47 (dd, *J* = 12.9, 8.2 Hz, 1H), 4.39 (dd, *J* = 12.6, 7.6 Hz, 1H), 4.30 (dd, *J* = 14.7, 7.8 Hz, 3H), 4.22 – 4.03 (m, 6H), 3.75 (dd, *J* = 118.2, 18.3 Hz, 6H), 3.30 (d, *J* = 60.2 Hz, 7H), 3.19 – 3.02 (m, 11H), 3.01 – 2.92 (m, 3H), 2.90 – 2.67 (m, 7H), 2.66 – 2.56 (m, 1H), 2.32 – 2.16 (m, 2H), 1.99 – 1.86 (m, 2H), 1.83 – 1.71 (m, 1H), 1.65 (s, 1H), 1.50 (t, *J* = 16.5 Hz, 1H), 1.39 (d, *J* = 6.6 Hz, 2H), 1.33 – 1.15 (m, 3H), 0.73 (dd, *J* = 17.1, 6.7 Hz, 6H) (Figure S65).

^{13}C NMR (126 MHz, DMSO-*d*6) δ 174.09, 174.06, 171.86, 171.71, 171.69, 171.50, 171.35, 170.90, 170.87, 170.84, 170.76, 170.73, 170.66, 163.70, 163.07, 158.50, 158.25, 157.99, 157.74, 136.07, 130.89, 130.63, 130.36, 130.10, 128.38, 126.41, 125.12, 124.24, 122.07, 120.48, 119.90, 118.11, 115.74, 113.37, 58.36, 57.93, 54.67, 53.92, 52.74, 52.35, 52.15, 50.92, 50.84, 50.42, 49.86, 49.72, 48.62, 40.11, 40.02, 39.94, 39.85, 39.77, 39.69, 39.52, 39.52, 39.35, 39.19, 39.02, 38.74, 37.66,

36.27, 35.92, 35.74, 30.42, 30.16, 26.77 (Figure S66).
HRMS (ESI) Calcd for $C_{93}H_{112}F_6N_{15}O_{24}$, $[M + H]^+$: 1936.7909, found: 1936.7914 (Figure S67).

Synthesis of **CF_3DEVDFFFK(Fmoc)-Gd (5)**
To a solution of compound **4** (96.8 mg, 0.05 mmol) in 10 mL CH_3CN/H_2O (v: v = 1:9) was carefully added a solution of 0.1 M NaOH to adjust pH value to ~6.0. Then, a solution of $GdCl_3$ (22.3 mg, 0.06 mmol) in 1 mL water was added. After stirring at r.t. for 10 min, the pH value of the reaction solution was further adjusted to ~6.0 using 0.1 M NaOH, and the reaction mixture was stirred at r.t. overnight. After the reaction, the reaction mixture was purified by HPLC to give **CF_3DEVDFFFK(Fmoc)-Gd** as a white solid (Yield: 92.4%, Purity: 98.8%, Figure S68).
HRMS (ESI) Calcd for $C_{93}H_{109}F_6GdN_{15}O_{24}$, $[M + H]^+$: 2091.6915, found: 2091.6916 (Figure S69).

1.5. Synthesis of CF_3DEVDC_2-Gd



Scheme S4. Synthetic routes of **CF_3DEVDC_2-Gd**. Reaction conditions: (a) TFA/DCM (v:v = 1:99), r.t., 15 min, 42.8%. (b) Compound **d**, HATU, DIPEA, r.t., 2 h, 72.6%; TFA/H_2O/TIPS (v:v:v = 95:2.5:2.5), r.t., 1 h, 81.5%. (c) $GdCl_3 \cdot 6H_2O$, CH_3CN/H_2O (v:v = 1:9), pH = 6.0, r.t., 12 h, 77.5%.

Synthesis of compound **2**
SPPS (2.0 g, 2 mmol 2-chlorotrityl chloride resin) was used to prepare the peptide CF_3-Asp (OtBu)-Glu (OtBu)-Val-Asp (OtBu)-OH-Resin (**1**). Then, compound **2** was obtained (756.7 mg, yield: 42.8%) after cleaving the peptide from the resin.
^{1}H NMR (500 MHz, DMSO-*d*6) δ 9.30 (d, *J* = 7.8 Hz, 1H), 8.53 (s, 2H), 8.33 (d, *J* = 7.3 Hz, 2H), 8.23 (d, *J* = 7.9 Hz, 1H), 7.77 (d, *J* = 8.9 Hz, 1H), 4.93 – 4.78 (m, 1H), 4.60 (dd, *J* = 13.8, 7.8 Hz, 1H), 4.39 – 4.29 (m, 1H), 4.22 (dd, *J* = 8.7, 6.7 Hz, 1H), 2.84 (dd, *J* = 16.1, 5.1 Hz, 1H), 2.74 – 2.62 (m, 2H), 2.55 (d, *J* = 7.9 Hz, 1H), 2.32 – 2.11 (m, 2H), 2.03 – 1.86 (m, 2H), 1.82 – 1.67 (m, 1H), 1.42 – 1.29 (m, 27H), 0.86 (dd, *J* = 18.5, 6.7 Hz, 6H) (Figure S70).
^{13}C NMR (126 MHz, DMSO-*d*6) δ 172.51, 172.21, 171.13, 170.94, 170.47, 169.72, 169.53, 163.90, 136.68, 131.35, 131.09, 130.83, 130.56, 128.75, 126.80, 125.42, 124.63, 122.46, 120.29, 80.62, 79.93, 65.36, 57.61, 52.44, 51.00, 49.07, 37.53, 31.71, 31.40, 28.04, 27.56, 19.50, 18.27, 15.56 (Figure S71).
HRMS (ESI) Calcd for $C_{27}H_{31}F_6N_4O_{12}$, $[M + H]^+$: 717.1843, found: 717.2000 (Figure S72).

Synthesis of compound **3**
Next, Compound **d** (135.1 mg, 0.22 mmol), HATU (76.5 mg, 0.2 mmol), and DIPEA (67.9 μL, 0.4

mmol) were added into compound **2** (176.8 mg, 0.2 mmol). After 2 h reaction, the mixture was purified by HPLC to afford an intermediate compound. Then, the intermediate compound was directly added into a solution of TIPSH (0.02 mmol) in TFA/DCM/CH_3CN (v: v: v = 95/2.5/2.5, 10 mL) stirred at r.t. for 1 h to remove the OtBu group completely. The solvent was then removed. Cold Et_2O (20 mL) was added and the precipitate was dried under vacuum and purified by HPLC to give intermediate compound **3** as a white solid.

^{1}H NMR (500 MHz, DMSO-*d*6) δ 9.32 – 9.22 (m, 1H), 8.52 (s, 2H), 8.45 – 8.26 (m, 3H), 8.20 (d, *J* = 7.2 Hz, 1H), 7.94 – 7.75 (m, 1H), 7.66 (dd, *J* = 30.2, 7.8 Hz, 1H), 4.80 (ddd, *J* = 13.8, 10.4, 6.9 Hz, 1H), 4.57 (dd, *J* = 13.5, 8.3 Hz, 1H), 4.47 (dd, *J* = 13.9, 7.3 Hz, 1H), 4.30 (dd, *J* = 12.6, 7.2 Hz, 1H), 4.07 (dt, *J* = 13.6, 7.3 Hz, 3H), 3.83 (s, 3H), 3.64 (s, 4H), 3.43 – 3.21 (m, 9H), 3.15 (d, *J* = 29.1 Hz, 10H), 2.83 (dd, *J* = 16.8, 4.3 Hz, 1H), 2.76 – 2.66 (m, 2H), 2.32 – 2.18 (m, 2H), 1.96 (ddd, *J* = 19.8, 13.4, 6.7 Hz, 2H), 1.82 – 1.71 (m, 1H), 0.82 (dd, *J* = 12.1, 6.7 Hz, 6H) (Figure S73).

^{13}C NMR (126 MHz, DMSO-*d*6) δ 174.09, 174.06, 171.86, 171.71, 171.69, 171.50, 171.35, 170.90, 170.87, 170.84, 170.76, 170.73, 170.66, 163.70, 163.07, 158.50, 158.25, 157.99, 157.74, 136.07, 130.89, 130.63, 130.36, 130.10, 128.38, 126.41, 125.12, 124.24, 122.07, 120.48, 119.90, 118.11, 115.74, 113.37, 58.36, 57.93, 54.67, 53.92, 52.74, 52.35, 52.15, 50.92, 50.84, 50.42, 49.86, 49.72, 48.62, 39.52, 38.74, 37.66, 36.27, 35.92, 35.74, 30.42, 30.16, 26.77, 26.64, 19.08, 19.01, 18.22, 17.98 (Figure S74).

HRMS (ESI) Calcd for $C_{45}H_{63}F_6N_{10}O_{18}$, $[M + H]^+$: 1145.4226, found: 1145.4222 (Figure S75).

Synthesis of compound **CF_3DEVDC_2-Gd (4)**

After that, a solution of compound **3** (114.7 mg, 0.1 mmol) in CH_3CN/H_2O (v: v = 1:9) was carefully added a solution of 0.1 M NaOH to adjust pH value to ~6.0. Then, a solution of $GdCl_3 \cdot 6H_2O$ (40.8 mg, 0.11 mmol) in 1 mL water was added. After stirring at r.t. for 10 min, the pH value of the reaction solution was further adjusted to ~6.0 using 0.1 M NaOH, and the reaction mixture was stirred at r.t. overnight. After the reaction, the reaction mixture was purified by HPLC to give **CF_3DEVDC_2-Gd** as a white solid (Yield: 77.5%, Purity: 98.14%, Figure S76).

HRMS (ESI) Calcd for $C_{45}H_{60}F_6GdN_{10}O_{18}$, $[M + H]^+$: 1300.3232, found: 1300.3233 (Figure S77).

1.6. Characterization of self-assembly process in solutions

For TEM analysis, **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) in 1 mL Tris buffer was incubated with caspase-3 (5 μg/mL) at 37 °C for 2 h. The aqueous solutions (~25 μL) was dropped onto a carbon-coated copper grid. The samples were then examined on a Hitachi S-4800 transmission electron microscope.

1.7. Determination of the sensitivity of CF_3DEVDFFFK(Fmoc)-Gd toward caspase-3

To evaluate the sensitivity of **CF_3DEVDFFFK(Fmoc)-Gd** toward caspase-3 by NMR relaxometry method, **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) was incubated with varying concentrations of caspase-3 (0, 0.01, 0.05, 0.1, 0.5, 1.0, 2.0, 4.0, and 5.0 μg/mL) in Tris buffer at 37 °C for 120 min and the T_1 value in each solution was then acquired by a series of inversion-prepared fast spin-echo scans on the 0.5 T NMR relaxometry (Bruker, Germany). The resulting $1/T_1$ were plotted to the concentrations of caspase-3, and a linear regression fitted from 0.5-2.0 μg/mL caspase-3 was obtained, affording the slope k. The limit of detection (LOD) was calculated from 3σ/k, where σ represents the standard deviation of 11 blank measurements.

1.8. Determination of the specificity toward caspase-3

The specificity toward caspase-3 *in vitro* was evaluated based on three modalities of HPLC, ^{19}F NMR, and T_1 value, respectively. First, **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) in Tris buffer was incubated with 2 μg/mL BSA, Gly, GSH, Glucose, Lysozyme, Trypsin or caspase-3 and the solutions were kept at 37 °C for 120 min. ^{19}F NMR experiments were performed on a Bruker 500 MHz NMR spectrometer under the temperature of 37 °C. Secondly, **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) in Tris buffer was incubated with 2 μg/mL BSA, Gly, GSH, Glucose, Lysozyme, Trypsin and caspase-3. The solutions were kept at 37 °C for 120 min. The T_1 value in each solution was then acquired by a series of inversion-prepared fast spin-echo scans on the 0.5 T NMR relaxometer (Bruker, Germany).

1.9. Stability evaluation of CF_3DEVDFFFK(Fmoc)-Gd under physiological conditions

The stability of **CF_3DEVDFFFK(Fmoc)-Gd** under physiological conditions was evaluated using HPLC, ^{19}F NMR and T_1 values, respectively. **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) was incubated in Tris buffer (pH = 7.4) containing 10% mice serum. The T_1 value in each solution was then acquired by a series of inversion-prepared fast spin-echo scans on the 0.5 T NMR relaxometer (Bruker, Germany). The data were recorded at 0 h, 6 h, 12 h, 24 h and 36 h. **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) was incubated in different pH of Tris buffer (pH = 3.0, 5.0, 7.0, 8.0, 9.0). The T_1 value in each solution was then acquired by a series of inversion-prepared fast spin-echo scans on the 0.5 T NMR relaxometer (Bruker, Germany).

2. *In Vitro* Studies of CF_3DEVDFFFK(Fmoc)-Gd

2.1. Cell culture

4T1 cell lines were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China) and cultured with the basic medium that contained 10% FBS, 100 U mL^{-1} penicillin, and 100 U mL^{-1} streptomycin under humidified air with 5% CO_2 at 37 °C. All agents were purchased from Boster Company (China) and filtered with a 0.2 μm sterile filter before incubation with cells.

2.2. Cell viability assays

The cytotoxicity of **CF_3DEVDFFFK(Fmoc)-Gd**, **CF_3DEVDC_2-Gd** or **FFFK(Fmoc)-C_2-Gd** to 4T1 cells was evaluated by Cell Counting Kit-8 (CCK8). In brief, cells (1×10^4 cells/well) in RPMI-1640 medium (supplemented with 10% FBS, 200 μL) were seeded in 96-well plate and incubated at 37 °C overnight. The medium was then changed and cells were treated with **CF_3DEVDFFFK(Fmoc)-Gd**, **CF_3DEVDC_2-Gd** or **FFFK(Fmoc)-C_2-Gd** at a serial of concentrations (0, 5, 10, 20, 40, 80, 100, and 300 μM) in FBS-free RPMI-1640 (100 μL) at 37 °C with 5% CO_2 and 95% humidity for 24 h. After the removal of the medium, FBS-free RPMI-1640 (90 μL) supplemented with CCK8 (10 μL, 1 × 100 T) was added to each well and incubated at 37 °C with 5% CO_2 and 95% humidity for 2 h. Absorbance at 450 nm was measured with a microplate reader and cell viability was calculated based on untreated wells. Each experiment was triplicated.

2.3. Measure the caspase-3/7 activity in cells

4T1 cells (1×10^6) were seeded onto cell culture dishes. After the cell grew to ~90% confluence, the medium was removed and 6 mL of fresh medium containing different concentrations of STS (2

μM) was added to the dishes and incubated for 4 h. Then, the medium was replaced with 4 mL of fresh medium with further incubation for 12 h. The medium was carefully removed and the cells were trypsinized, centrifuged (1200 rpm, 4 min), and washed with PBS twice. The obtained cell pellets were sonicated using an ultrasonic homogenizer in an ice bath for 60 s. The cell homogenates were centrifuged (12000 rpm) for 3 min, and the supernatant was then collected. To evaluate caspase-3 activity, 50 μL of the cell lysate solution was added to a 96-well black plate and 50 μL of caspase-3 assay buffer containing 20 μM Ac-DEVD-AMC was added. The solutions were incubated at 37 ^{o}C for 2 h, and the corresponding fluorescence intensity at 460 nm (λ_{ex} = 380 nm) was recorded on a microplate reader.

2.4. Cellular uptake

Time-dependent cellular uptake was determined in 4T1 cells plated at a density of 50,000 cells per well in a 24-well plate. **CF_3DEVDFFFK(Fmoc)-Gd** was dissolved in media at 200 μM and incubated with cells for different times (0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 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.

2.5. Bio-transmission electron microscopy (Bio-TEM) measurement

4T1 cells (1×10^6) were seeded onto cell culture dishes. After the cell grew to ~90% confluence, the medium was removed and 6 mL of fresh medium containing STS (2 μM) was added to the dishes and incubated for 4 h, and then incubated with **CF_3DEVDFFFK(Fmoc)-Gd** (500 μM) in FBS free RPMI-1640 for 8 h. Then, 4T1 cells were collected and washed with PBS 3 times, the treated cells were fixed with 4% glutaraldehyde at 4 °C overnight. After immobilization in 1% OsO_4 for 1 h at room temperature, the cells were dehydrated using ethanol with graded concentrations and then embedded in epoxy resin. An ultramicrotome (Leica Microanalysis) was used to prepare ultrathin sections of the cells. Then, the obtained cell sections were stained with uranyl acetate/lead citrate and imaged under the transmission electron microscope.

3. *In Vivo* Imaging with CF_3DEVDFFFK(Fmoc)-Gd

3.1 Animals and cancer model mice

All experimental protocols involving animals were approved by the Animal Welfare and Research Ethics Committee at Innovation Academy for Precision Measurement Science and Technology, Chinese Academy of Sciences (APM21018T). To establish tumors in six-week-old BALB/c female mice, two million 4T1 cells were suspended in 50 μL PBS were injected subcutaneously into the selected positions of BALB/c mice. Tumors were grown until a single aspect was 0.5- 0.7 cm (approximately 7- 10 days). Then, treatment that consisted of 5 mg kg^{-1} DOX or saline was initiated through tail vein injection, once every two days for a total of three times. Mouse body weight and tumor size (width and length by caliper) were measured every other day. Tumor volumes were calculated assuming an ellipsoid shape with the formula (length × $width^2$)/ 2.

3.2. Detection of caspase-3 activity in saline- and DOX-treated 4T1 tumor lysates

BALB/c female mice with 4T1 tumor xenograft were treated with saline, or DOX (5 mg kg^{-1}) according to the protocol for the chemotherapy mouse model. After treatment, the mice were sacrificed and the tumors were resected and Tris buffer (1g tissue/mL buffer) were added. The tumor tissues were fully grinded at 4 °C for 2 min, followed by sonicating using an ultrasonic homogenizer in ice bath for 60 s. The tumor homogenates were centrifuged (14000g) at 4 °C for 30 min and the supernatant was then collected. To evaluate caspase-3 activity, 50 μL of the tumor lysate solution was added to a 96-well black plate and 50 μL of caspase-3 assay buffer containing 20 μM Ac-DEVD-AMC was added. The solutions were incubated at 37 °C for 2 h, and the corresponding fluorescence intensity at 460 nm (λ_{ex} = 380 nm) was recorded on a microplate reader.

3.3. Biodistribution studies

4T1 tumor-bearing mice were i.v. injected with **CF_3DEVDFFFK(Fmoc)-Gd**, **CF_3DEVDC_2-Gd** or Gd-DTPA (0.084 mmol kg^{-1} Gd^{3+}) in 200 μL PBS (n = 3 per group). After being sacrificed at 30 min, 2 h, 6 h, 12 h tumors and major organs including liver, kidneys, heart, lung, and spleen were collected and weighed. The tissues were cut into small pieces and digested with concentrated HNO_3 under heating at 120 ºC overnight. The residue in each organ was then diluted with 5 mL 2% HNO_3 solution, and the concentration of Gd^{3+} was determined by ICP-MS. The %ID/g was also calculated for comparison.

3.4. H&E staining

The major organs (heart, liver, spleen, lung, and kidney) were dissected and fixed in 4% paraformaldehyde and subjected to hematoxylin and eosin (H&E) staining assays.

4. Supplementary Figures

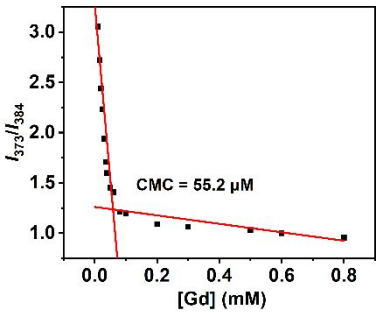


Figure S1. The CMC of **FFFK(Fmoc)-C_2-Gd**. The CMC was detected using pyrene as a fluorescent indicator (excitation wavelength, 334 nm). The ratio of fluorescence intensity at 384 nm and 373 nm from the pyrene was plotted against the Gd^{3+} concentration to calculate the CMC.

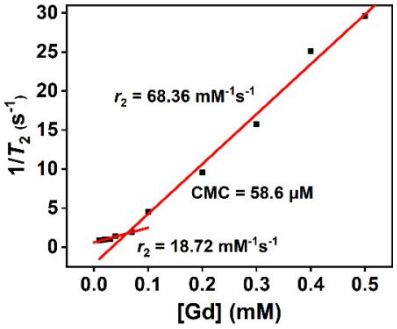


Figure S2. The plots of $1/T_2$ versus concentration of **FFFK(Fmoc)-C_2-Gd** in Tris-HCl (pH 7.4, 0.5 T) to determine the CMC of **FFFK(Fmoc)-C_2-Gd**. The r_2 was determined from the slope of each linear fit. The CMC (58.6 μM by T_2) of **FFFK(Fmoc)-C_2-Gd** was obtained from the intersection of the two linear fit lines.

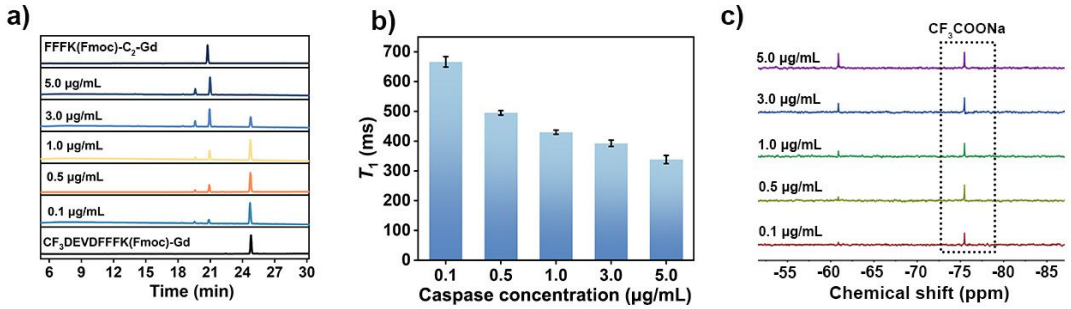


Figure S3. HPLC traces (a), T_1 values (b) and ^{19}F NMR (c) of **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) after being incubated with different concentrations of caspase-3 for 2 h at 37 °C. T_1 values are mean ± standard deviation (SD, n = 3).

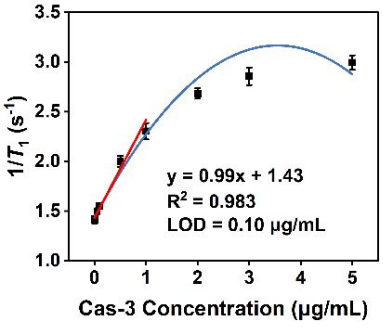


Figure S4. $1/T_1$ values of **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) incubated with different concentrations of caspase-3 for 2 h. The linear fitting curve of $1/T_1$ values with the concentration of caspase-3 from 0.05-2.0 μg/mL. The detection limit was found to be 0.10 μg/mL, using a 3σ/k method.

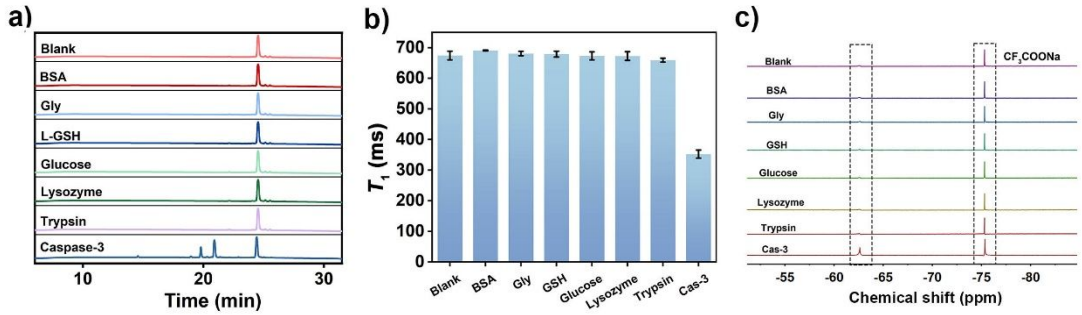


Figure S5. HPLC traces (a), T_1 values (b) and ^{19}F NMR (c) of **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) after being incubated with BSA, Gly, GSH, Glucose, Lysozyme, Trypsin, and caspase-3 (2 μg/mL) for 2 h at 37 °C. T_1 values are mean ± standard deviation (SD, n=3).

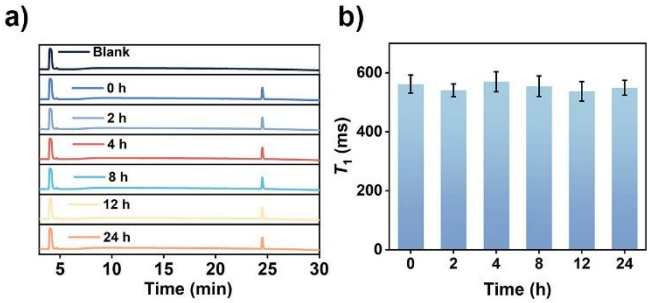


Figure S6. Stability test of **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM). Time-dependent HPLC traces (a) and T_1 values (b) of **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) incubated in RPMI-1640 containing 10% FBS for 24 h. T_1 values are mean ± standard deviation (SD, n=3).

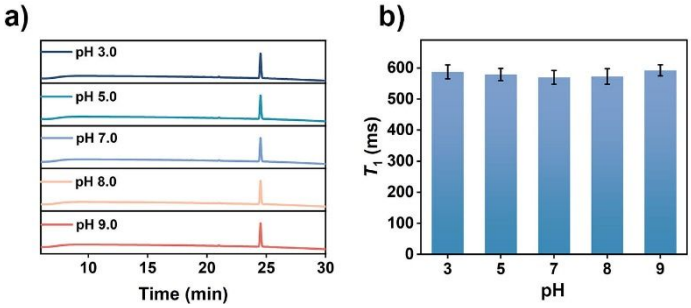


Figure S7. Stability test of **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM). pH-dependent HPLC traces (a) and T_1 values (b) of **CF_3DEVDFFFK(Fmoc)-Gd** (100 μM) in PBS Buffer for 8 h. T_1 values are mean ± standard deviation (SD, n=3).

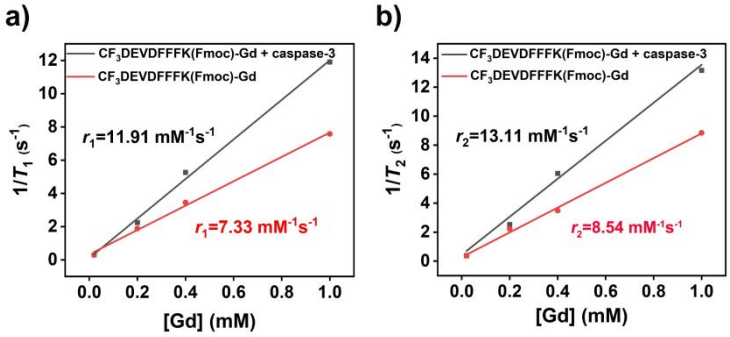


Figure S8. Plots of $1/T_1$ and $1/T_2$ vs Gd^{3+} concentration to determine the r_1 (a) and r_2 (b) of **CF_3DEVDFFFK(Fmoc)-Gd** on 9.4 T at 37 °C.

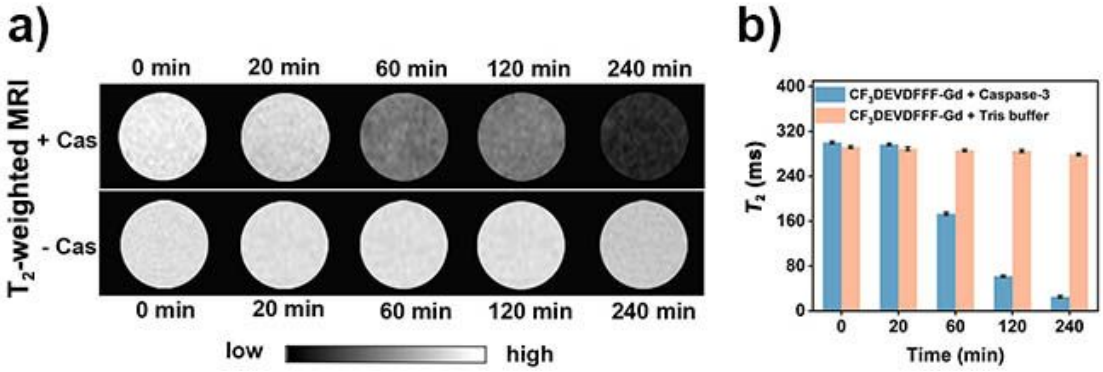


Figure S9. (a) Time course of T_2-weighted MRI of **CF_3DEVDFFFK(Fmoc)-Gd** with or without caspase-3 in Tris buffer (pH 7.4) at 9.4 T. (b) T_2 values of **CF_3DEVDFFFK(Fmoc)-Gd** incubated with or without caspase-3 for a different time. T_2 values are mean ± standard deviation (SD, n=3).

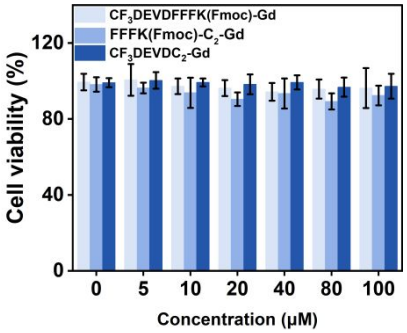


Figure S10. The cell viability test. 4T1 cells were incubated with **CF_3DEVDFFFK(Fmoc)-Gd**, **CF_3DEVDC_2-Gd** and **FFFK(Fmoc)-C_2-Gd** at 0, 5, 10, 20, 40, 80 and 100 μM for 24 h, and the cell viability was determined by CCK8 assay. Data bars show mean ± standard deviation (SD, n = 6 technical replicates).

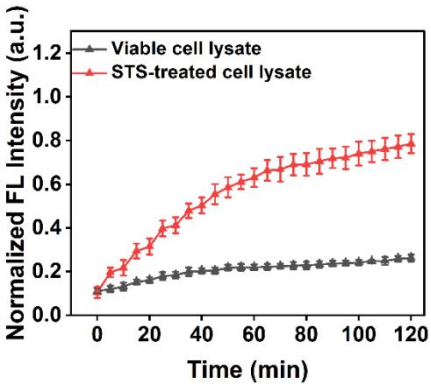


Figure S11. Plots of the normalized fluorescence (FL) intensity of Ac-DEVD-AMC (10 μM) incubated with viable cell lysate (black) or STS-treated apoptotic cell lysate (red) at 37 °C for 0-120 min. Protein concentrations of viable and apoptotic cell lysates were determined by the BCA protein calibration kit and used at the same level (2.0 mg/mL). FL intensity are mean ± standard deviation (SD, n=3).

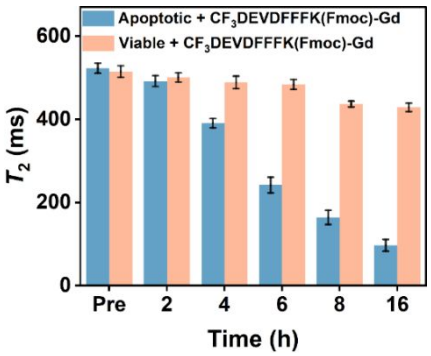


Figure S12. T_2 values of **CF_3DEVDFFFK(Fmoc)-Gd** incubated with viable cell (orange) or STS-induced apoptotic cell (blue). T_2 values are mean ± standard deviation (SD, n=3).

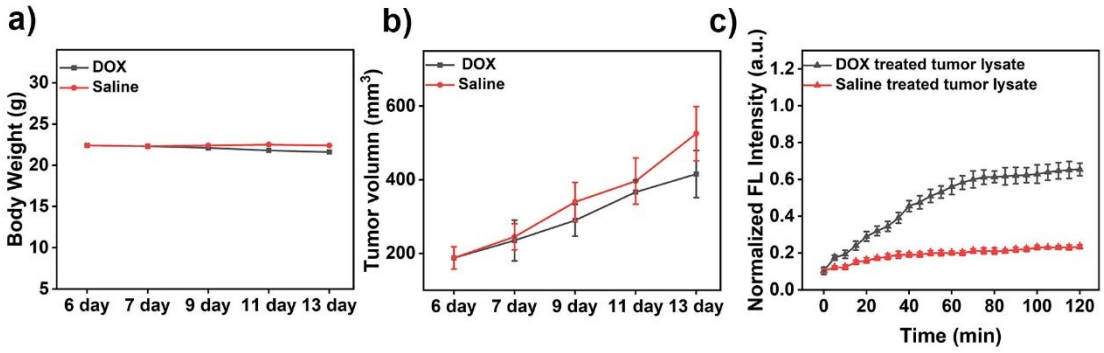


Figure S13. (a) Longitudinal monitoring of body weight change of Saline-treated (red) or DOX-treated (black) mice. (b) Longitudinal monitoring of tumor size change of Saline-treated (red) or DOX-treated (black) mice. (c) Plots of the fluorescence intensity of Ac-DEVD-AMC (10 μM) incubated with Saline-treated tumor lysate (red) or DOX-treated tumor lysate (blue) at 37 °C for 0-120 min vs. incubation time. Values represent mean ± standard deviation (SD) (n = 3).

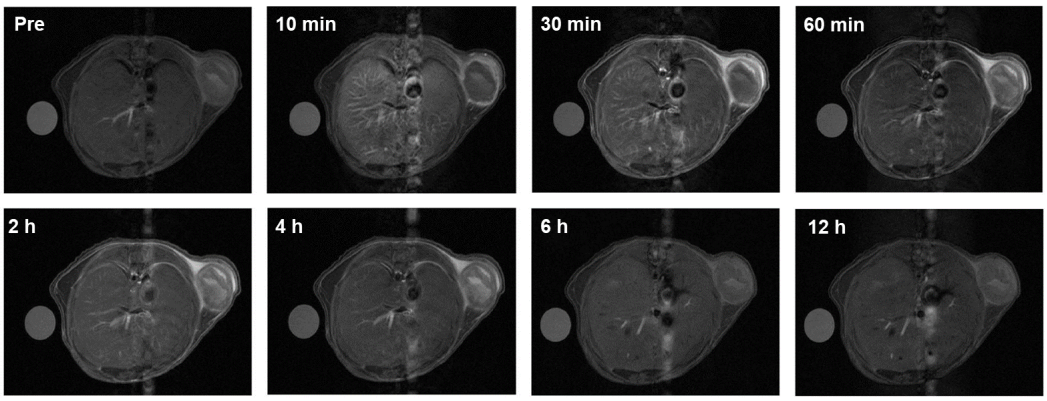


Figure S14. T_1-weighted MR images of saline-treated 4T1 tumors in living mice before (Pre) and at 10 min, 30 min, 60 min, 2 h, 4 h, 6 h, and 12 h post *i.v.* injection of **CF_3DEVDFFFK(Fmoc)-Gd** (0.084 mmol/kg).

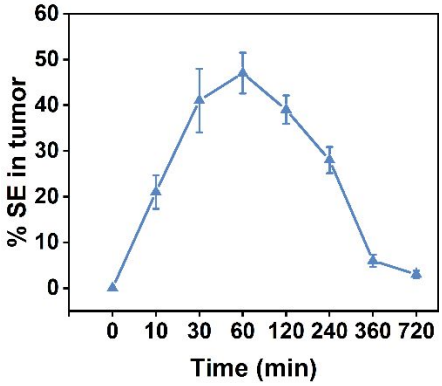


Figure S15. Quantification of %SE in tumors before (Pre) and at 10 min, 30 min, 60 min, 2 h, 4 h, 6 h and 12 h post i.v. injection of **CF_3DEVDFFFK(Fmoc)-Gd** (0.084 mmol/kg). Values are mean ± SD (n = 3).

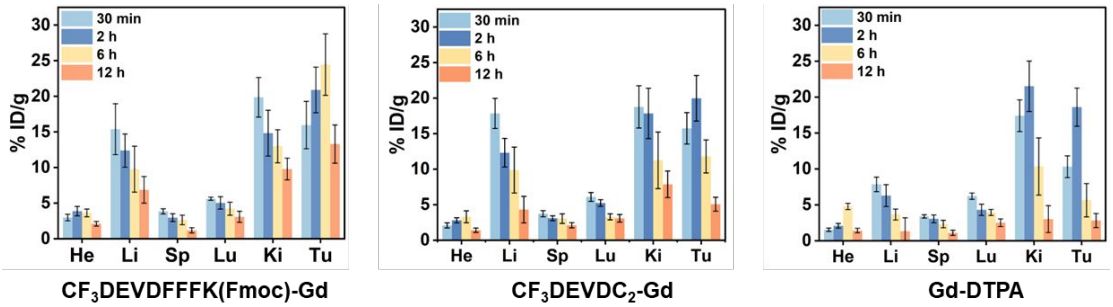


Figure S16. Biodistribution (% ID/g) of **CF_3DEVDFFFK(Fmoc)-Gd**, **CF_3DEVDC_2-Gd** and Gd-DTPA in 4T1 tumors and main organs (He: heart, Li: liver, Sp: spleen, Lu: lung, Ki: kidney, Tu: tumor) at 30 min, 2 h, 6 h, 12 h after *i.v.* injection into mice (0.084 mmol kg^{-1} Gd^{3+}). The amount of Gd^{3+} in tumors and main organs were determined by ICP-MS. Values represent mean ± standard deviation (SD) (n = 3).

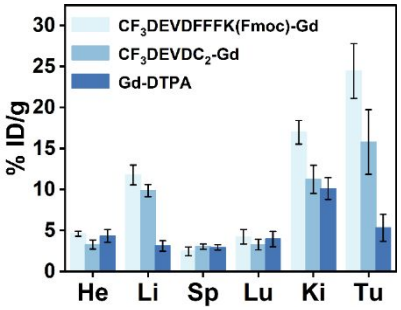


Figure S17. Biodistribution (% ID/g) of **CF_3DEVDFFFK(Fmoc)-Gd**, **CF_3DEVDC_2-Gd** and Gd-DTPA in 4T1 tumors and main organs (H: heart, Li: liver, Sp: spleen, Lu: lung, Ki: kidneys, Tu: tumor) at 2 h after *i.v.*injection into mice (0.084 mmol kg^{-1} Gd^{3+}). The amount of Gd^{3+} in tumors and main organs were determined by ICP-MS. Values represent mean ± standard deviation (SD) (n = 3).

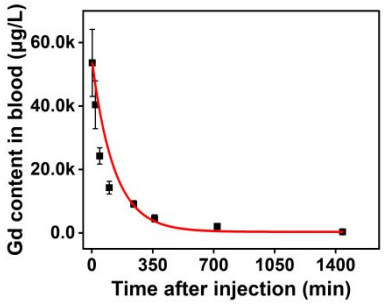


Figure S18. Half-life time in normal mice after tail vein injection of the **CF_3DEVDFFFK(Fmoc)-Gd** (0.084 mmol kg^{-1} Gd^{3+}). Blood Gd^{3+} levels were measured by ICP-MS. Values represent mean ± standard deviation (SD) (n = 4).

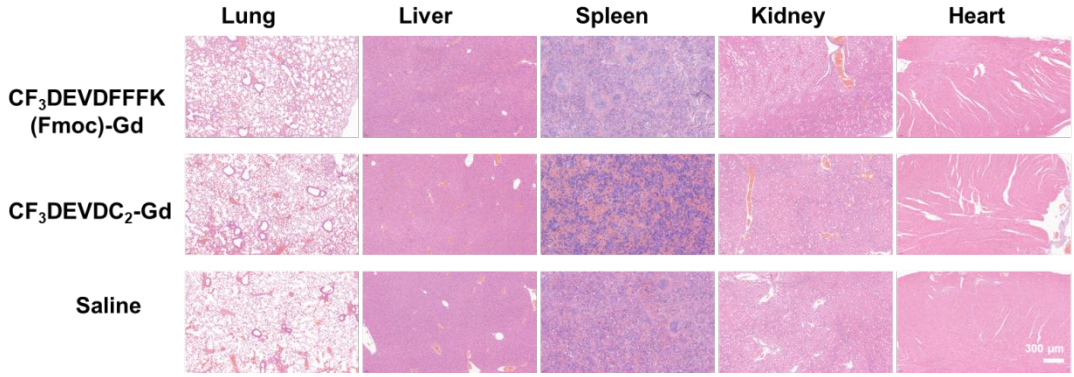


Figure S19. H&E staining of main organs resected from healthy mice after intravenous injection of **CF_3DEVDFFFK(Fmoc)-Gd** (0.084 mmol/kg) for 48 h. Scale bars = 300 μm.

5. Supplementary Table

Table S1. HPLC conditions for the analysis of all compounds. [a]

Time (min)	Flow (mL/min.)	H_2O (0.1 % TFA) %	CH_3CN (0.1% TFA) %

0	1.0	95	5
30	1.0	5	95
35	1.0	5	95

[a] HPLC was operated on the reversed-phase C18 (Waters C18, 5 μm, 4.6 × 250 mm) column.

Table S2. HPLC conditions for purification of **FFF-C_2-Gd, FFF-C_4-Gd, FFF-C_5-Gd**.[a]

Time (min)	Flow (mL/min)	H_2O (0.1 % TFA) %	CH_3CN (0.1% TFA) %
0	8.0	95	5
30	8.0	20	80
32	8.0	5	95
35	8.0	5	95

[a] HPLC was operated on the reversed-phase C18 (E-SCI, 10 μm, 21.12 × 250 mm) column.

Table S3. HPLC conditions bio-transmission electron microscopy for purification of **CF_3DEVDFFFK(Fmoc)-Gd, CF_3DEVDFFFC_2-Gd, FFFK(Fmoc)-C_2-Gd**.[a]

Time (min)	Flow (mL/min)	H_2O (0.1 % TFA) %	CH_3CN (0.1% TFA) %
0	8.0	95	5
5	8.0	70	30
35	8.0	2	98
40	8.0	2	98

[a] HPLC was operated on the reversed-phase C18 (E-SCI, 10 μm, 21.12 × 250 mm) column.

6. NMR and MS spectra

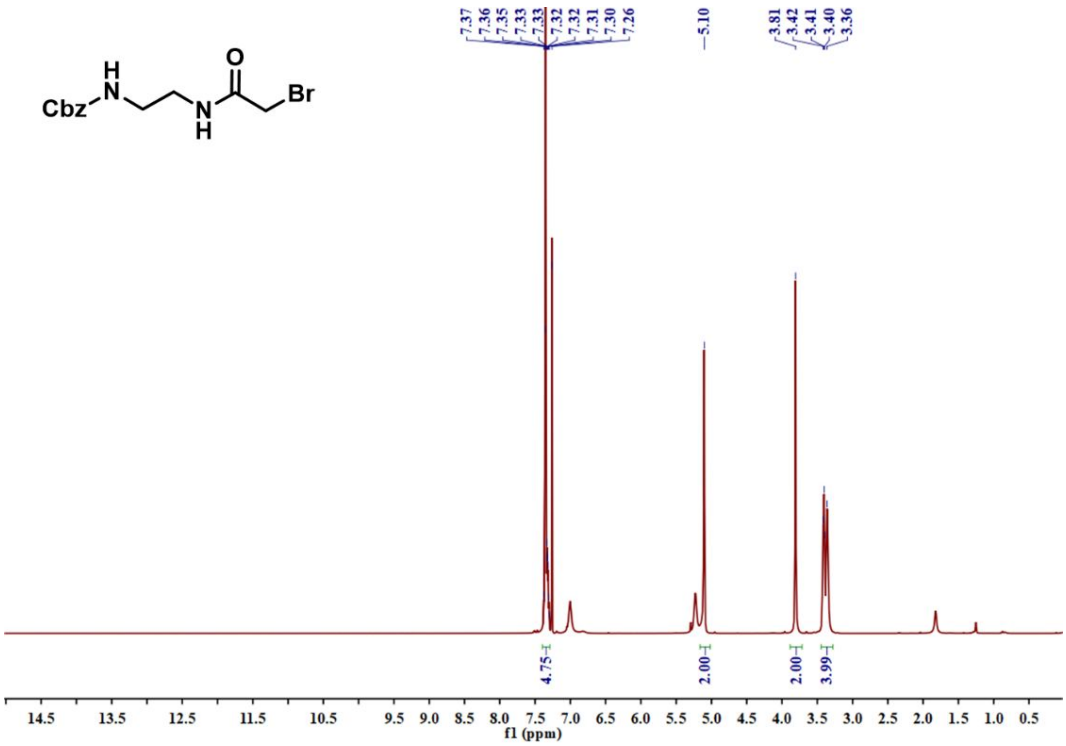


Figure S20. ^{1}H-NMR spectrum of compound **2a** in $CDCl_3$ at room temperature (500 MHz)

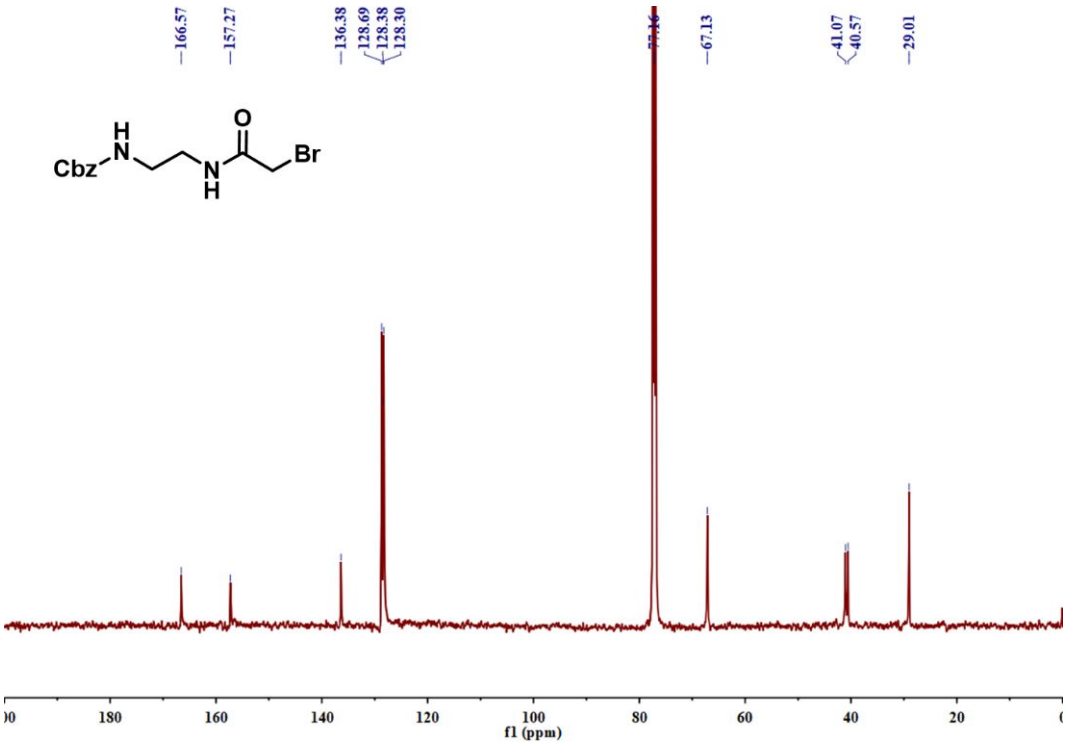


Figure S21. ^{13}C-NMR spectrum of compound **2a** in $CDCl_3$ at room temperature (126 MHz)

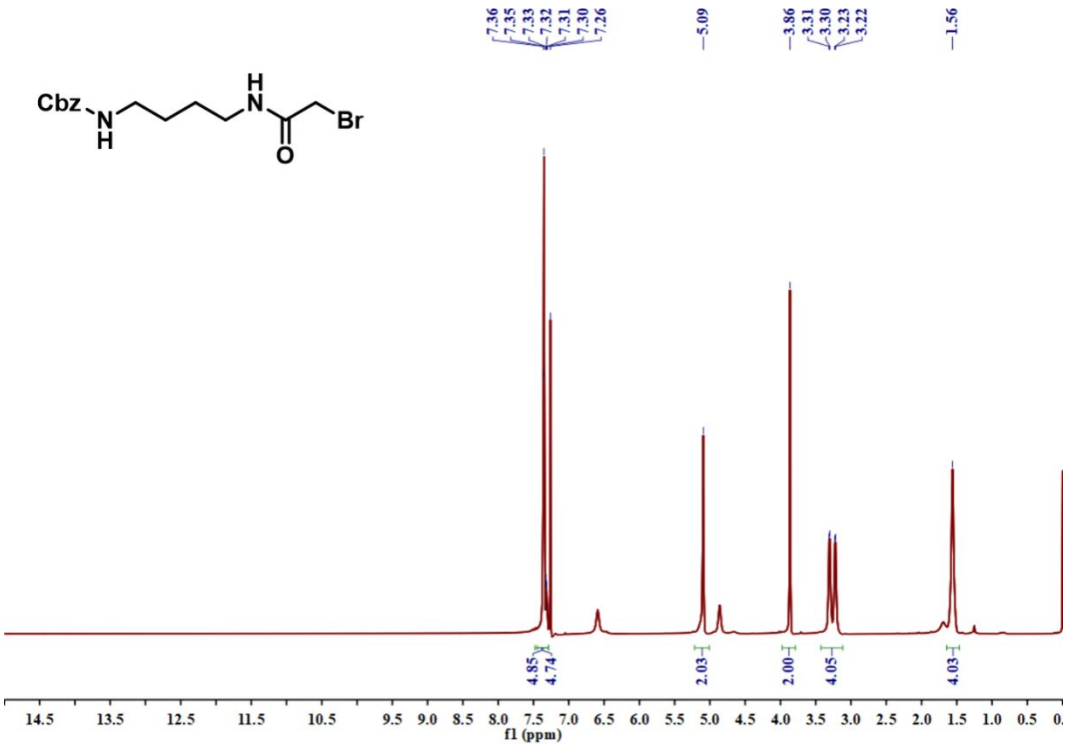


Figure S22. ^{1}H-NMR spectrum of compound **2b** in $CDCl_3$ at room temperature (500 MHz)

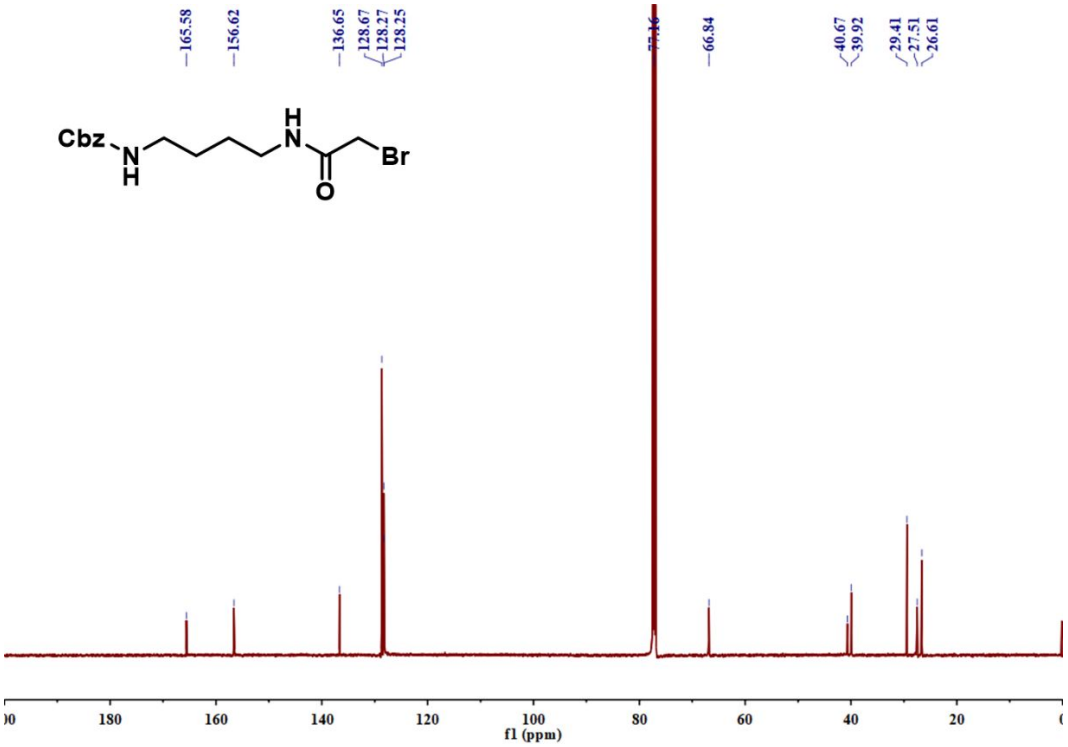


Figure S23. ^{13}C-NMR spectrum of compound **2b** in $CDCl_3$ at room temperature (126 MHz)

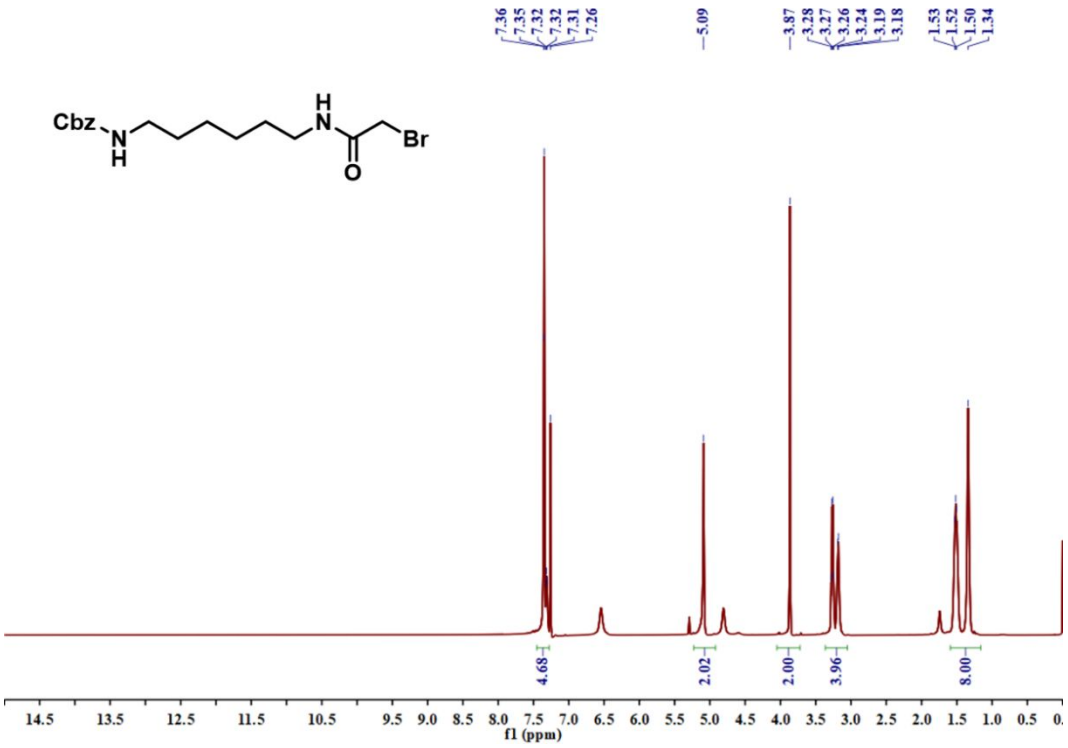


Figure S24. ^{1}H-NMR spectrum of compound **2c** in $CDCl_3$ at room temperature (500 MHz)

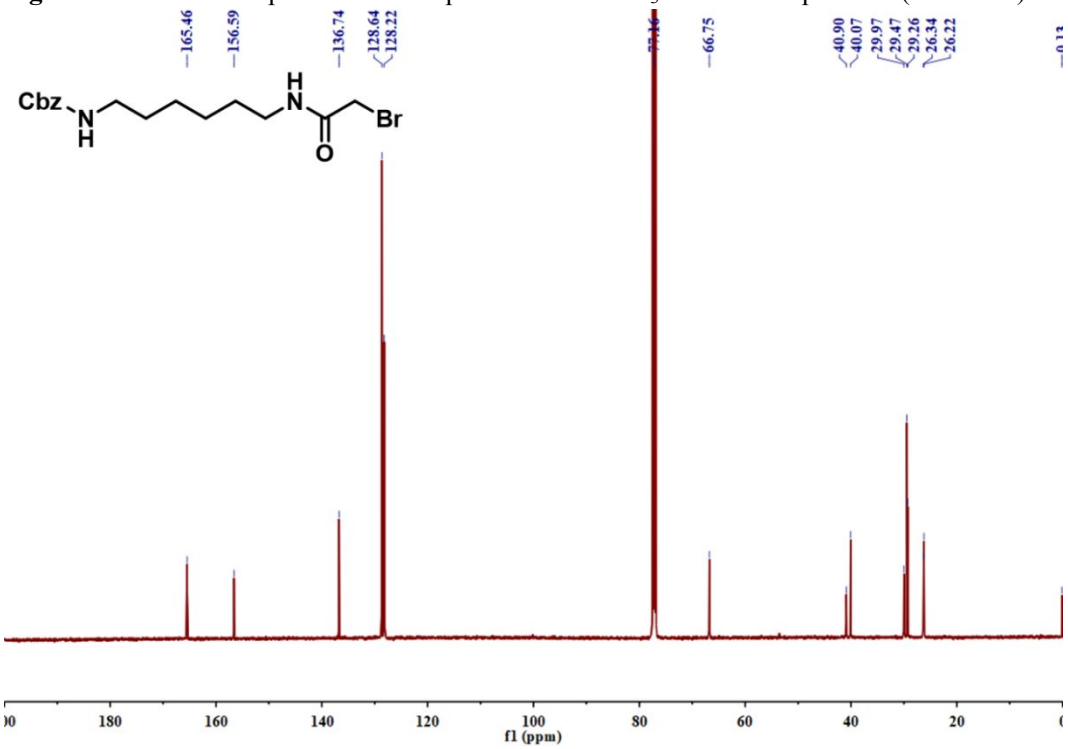


Figure S25. ^{13}C-NMR spectrum of compound **2c** in $CDCl_3$ at room temperature (126 MHz)

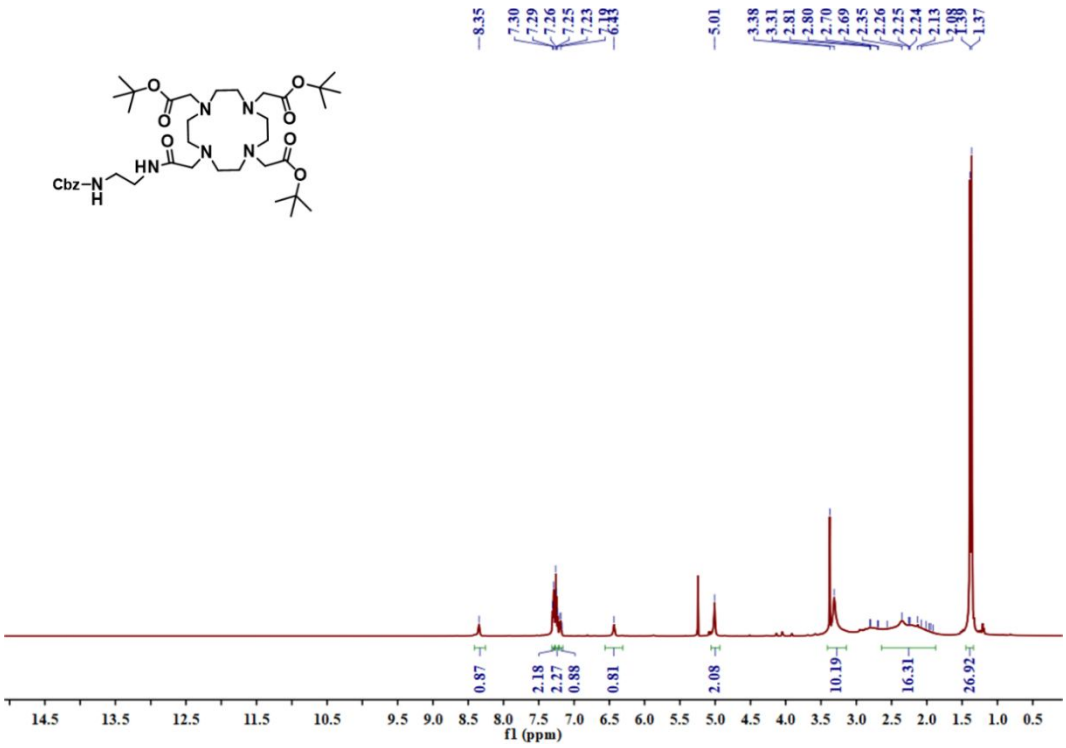


Figure S26. ^{1}H-NMR spectrum of compound **3a** in $CDCl_3$ at room temperature (500 MHz)

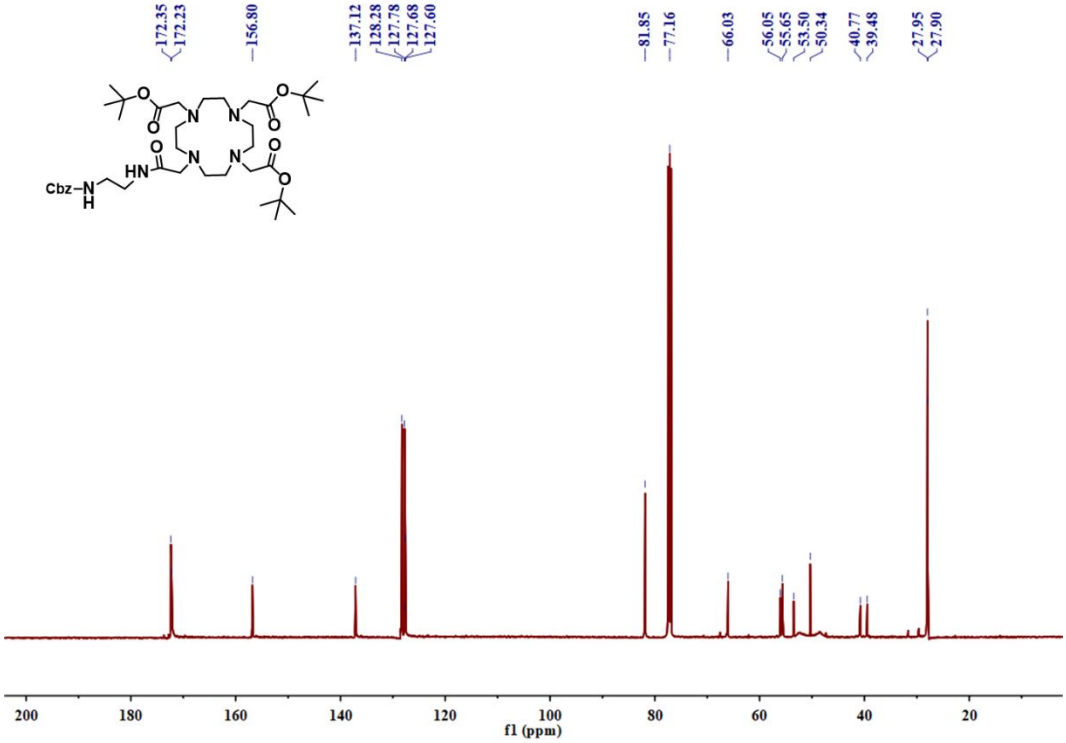


Figure S27. ^{13}C-NMR spectrum of compound **3a** in $CDCl_3$ at room temperature (126 MHz)

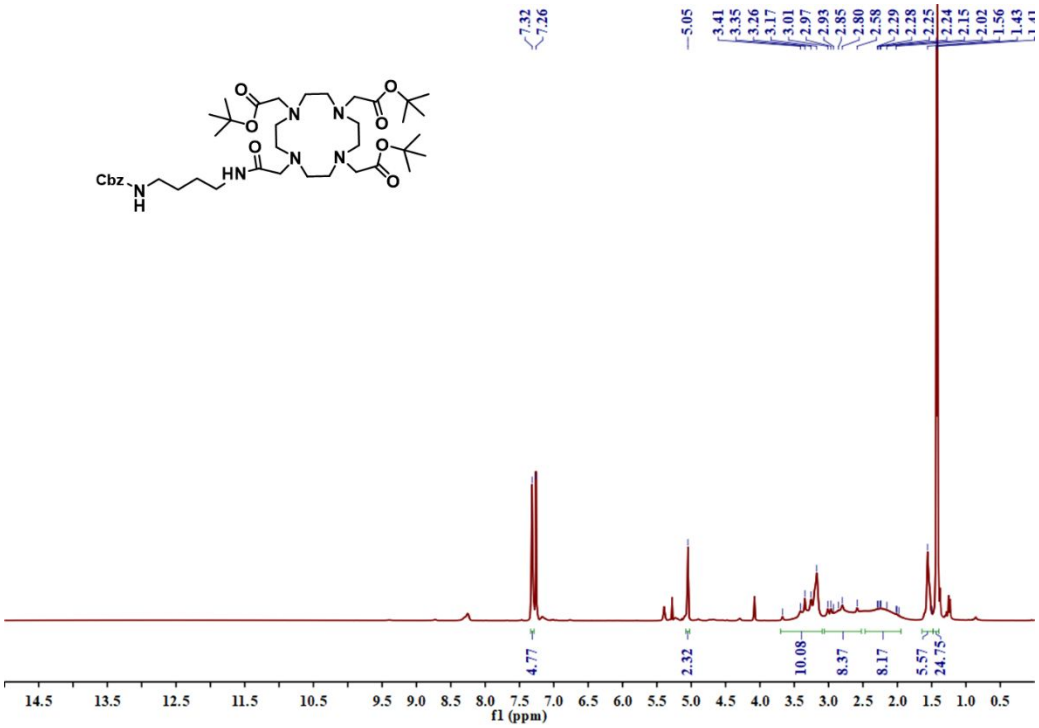


Figure S28. ^{1}H-NMR spectrum of compound **3b** in $CDCl_3$ at room temperature (500 MHz)

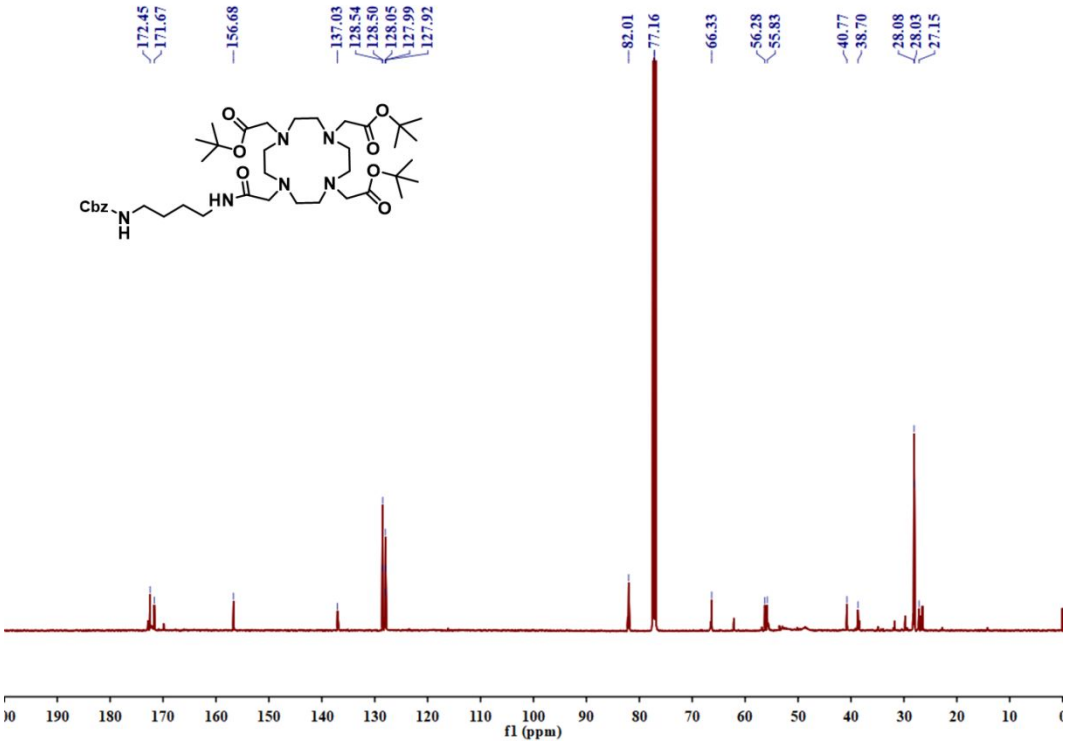


Figure S29. ^{13}C-NMR spectrum of compound **3b** in $CDCl_3$ at room temperature (126 MHz)

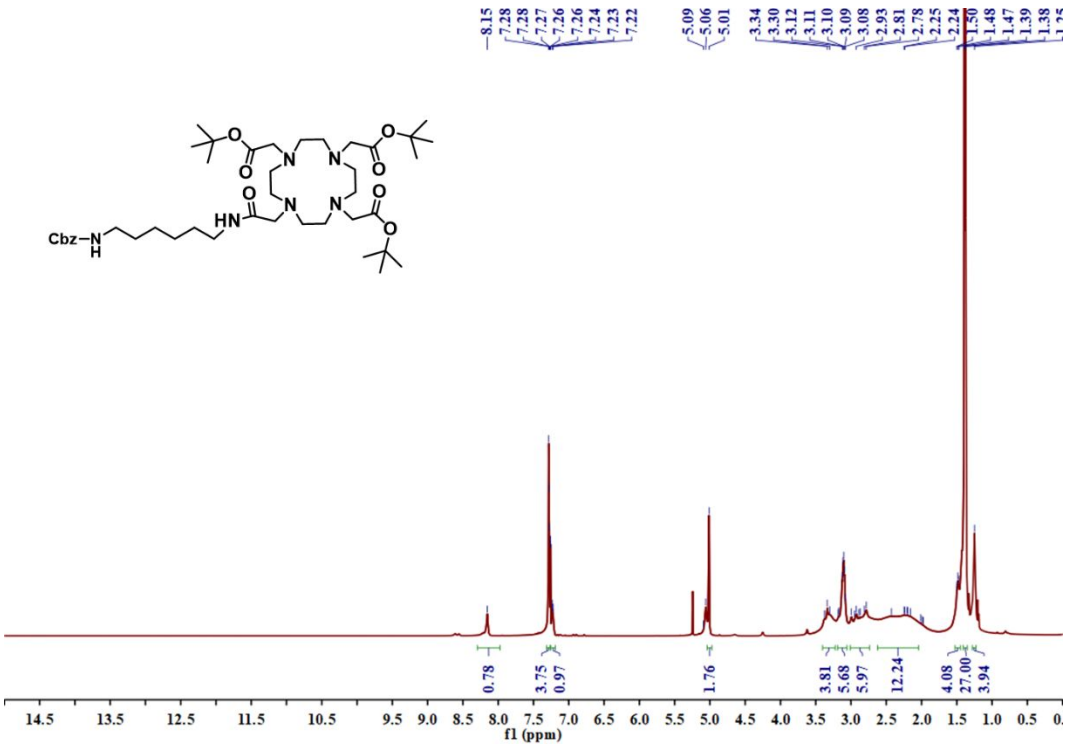


Figure S30. ^{1}H-NMR spectrum of compound **3c** in $CDCl_3$ at room temperature (500 MHz)

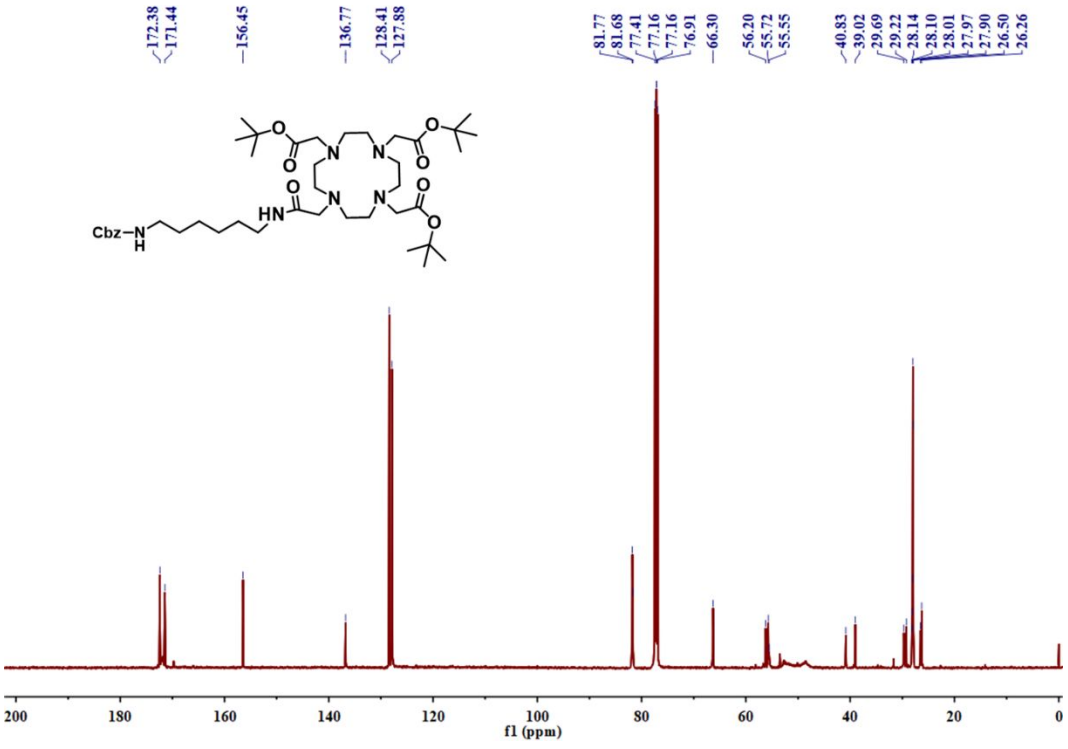


Figure S31. ^{13}C-NMR spectrum of compound **3c** in $CDCl_3$ at room temperature (126 MHz)

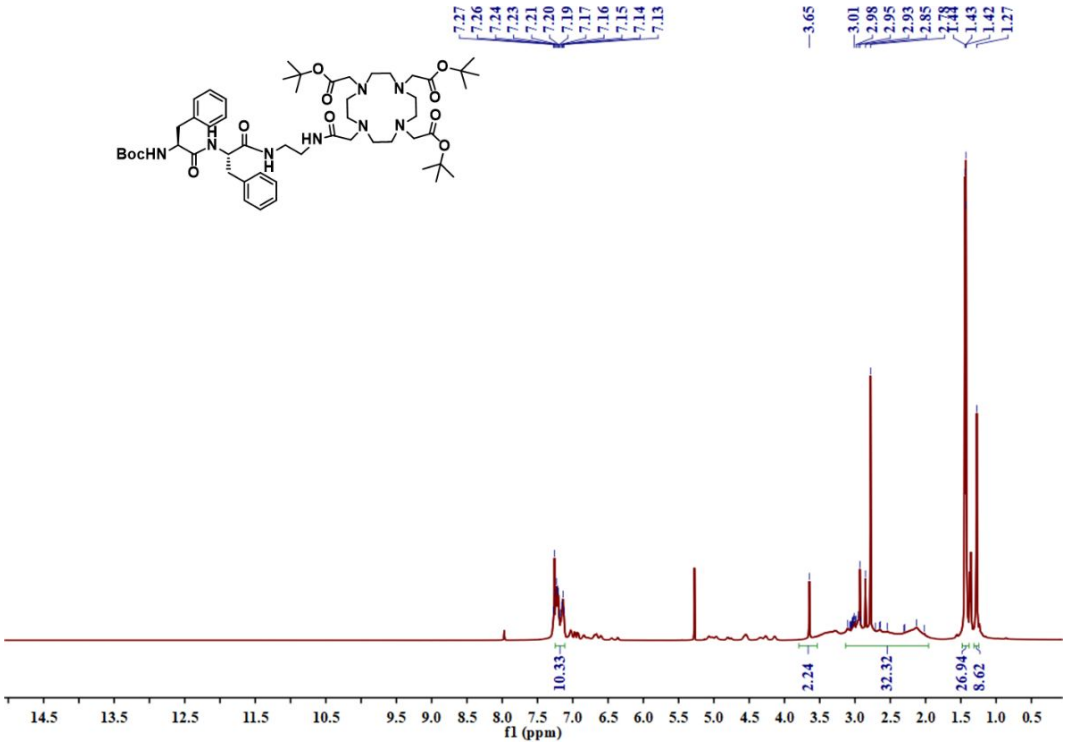


Figure S32. ^{1}H-NMR spectrum of compound **5a** in $CDCl_3$ at room temperature (500 MHz)

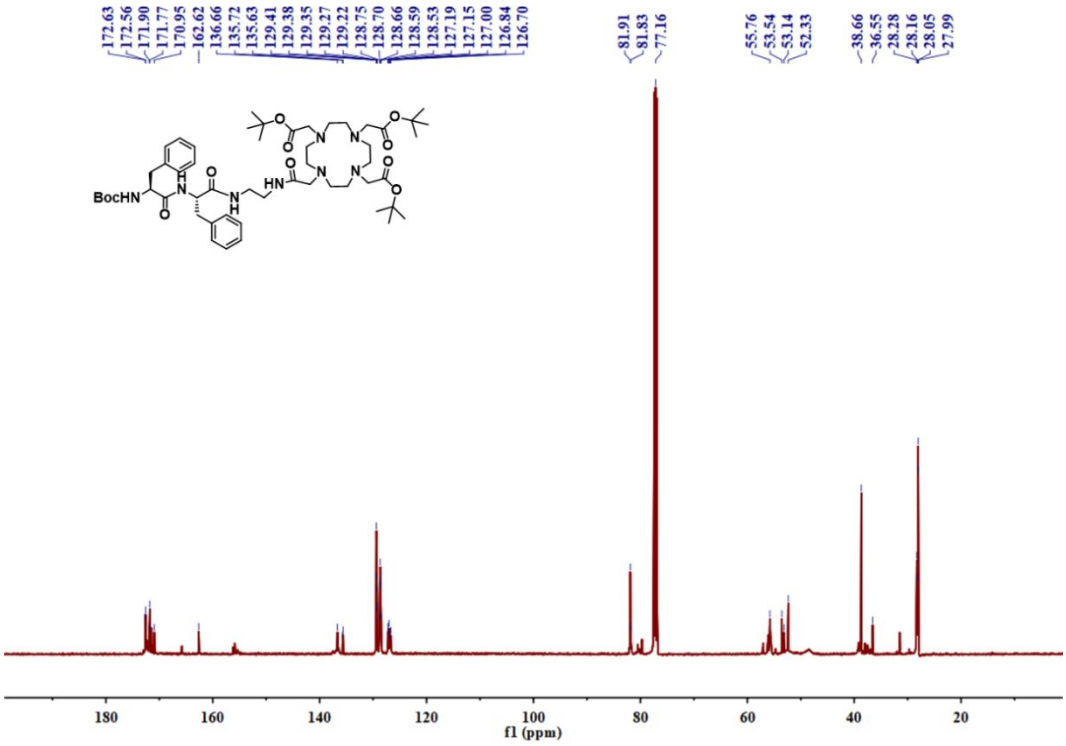


Figure S33. ^{13}C-NMR spectrum of compound **5a** in $CDCl_3$ at room temperature (126 MHz)

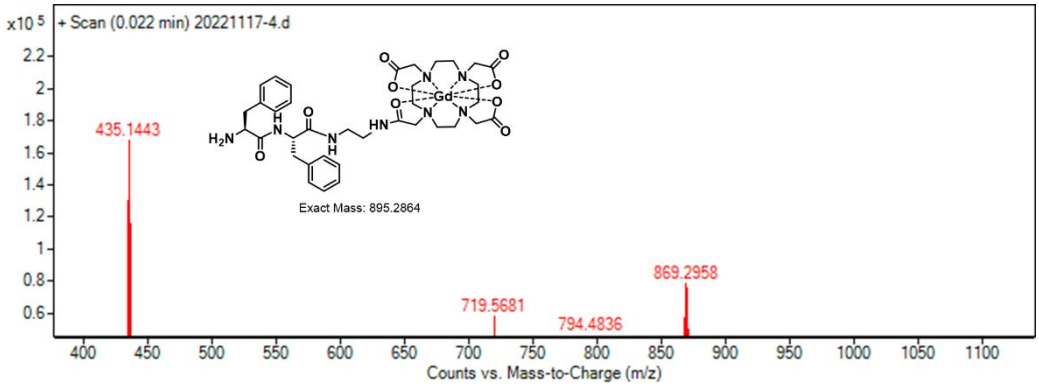


Figure S34. HRMS spectrum of compound **6a** (FF-C_2-Gd)

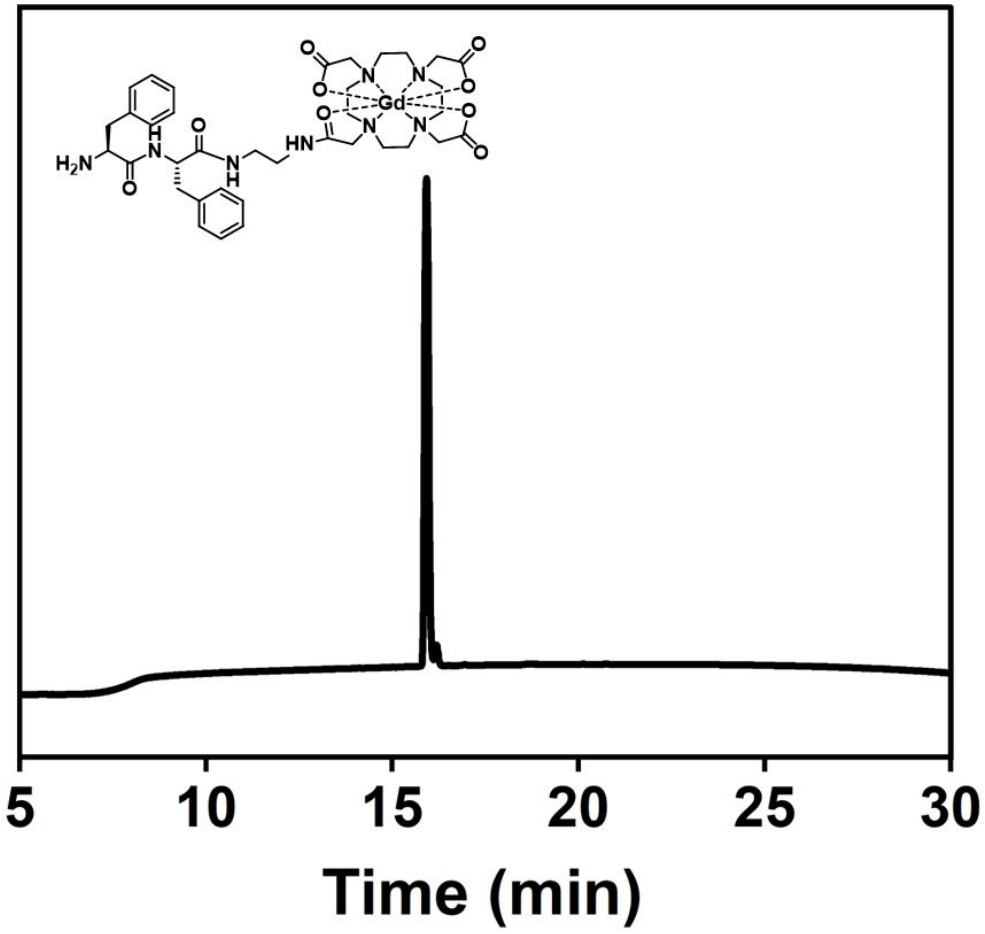


Figure S35. HPLC traces of compound **6a** (FF-C_2-Gd)

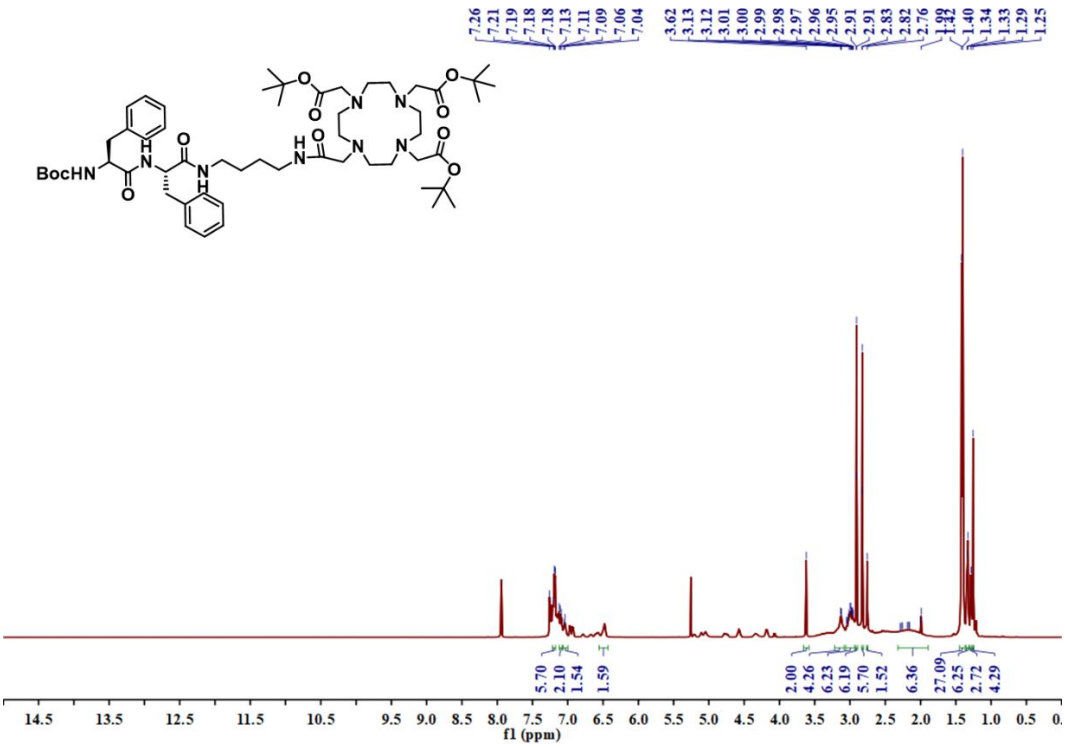


Figure S36. ^{1}H-NMR spectrum of compound **5b** in $CDCl_3$ at room temperature (500 MHz)

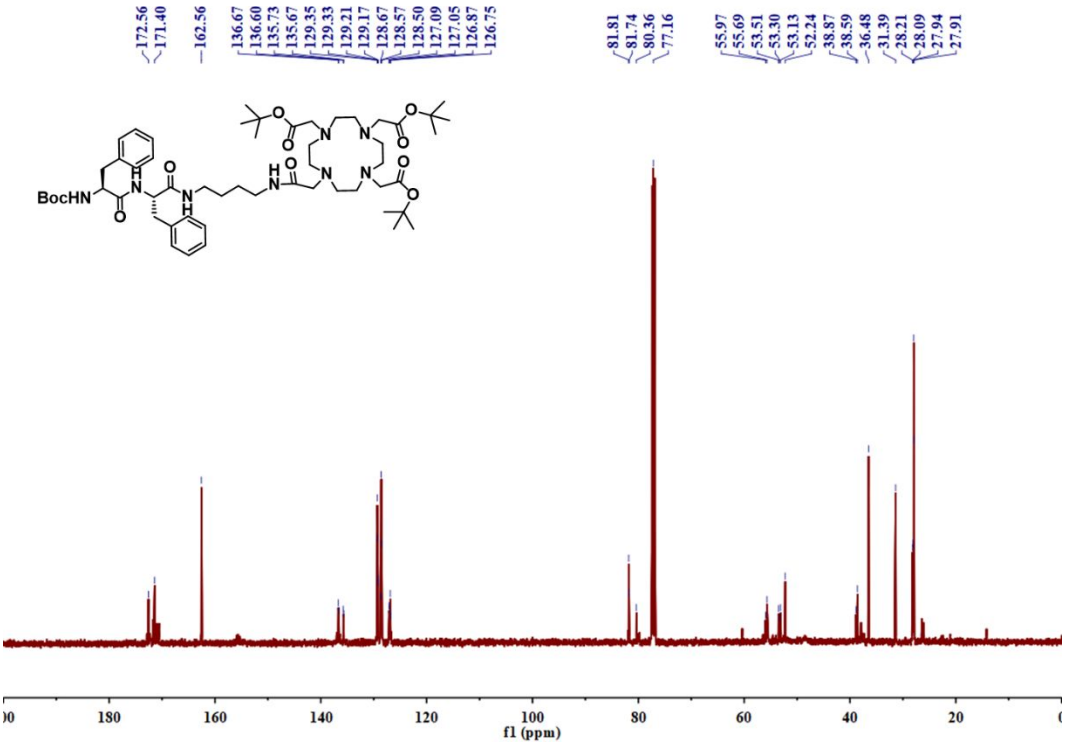


Figure S37. ^{13}C-NMR spectrum of compound **5b** in $CDCl_3$ at room temperature (126 MHz)

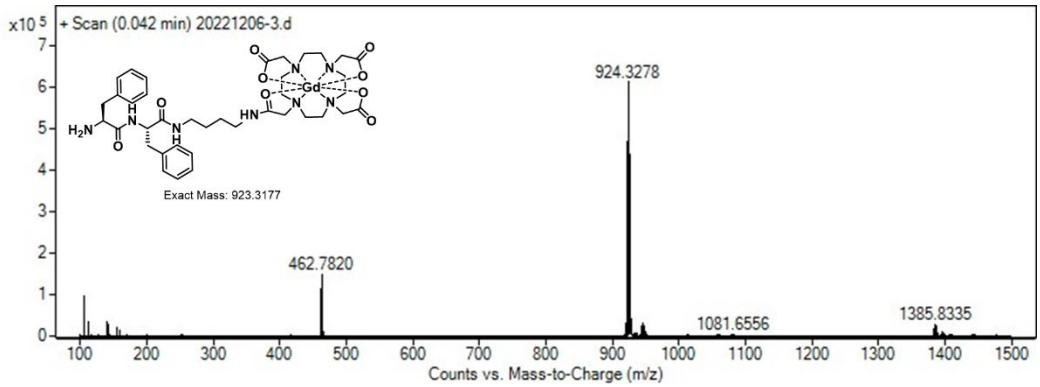


Figure S38. HRMS spectrum of compound **6b** (FF-C_4-Gd)

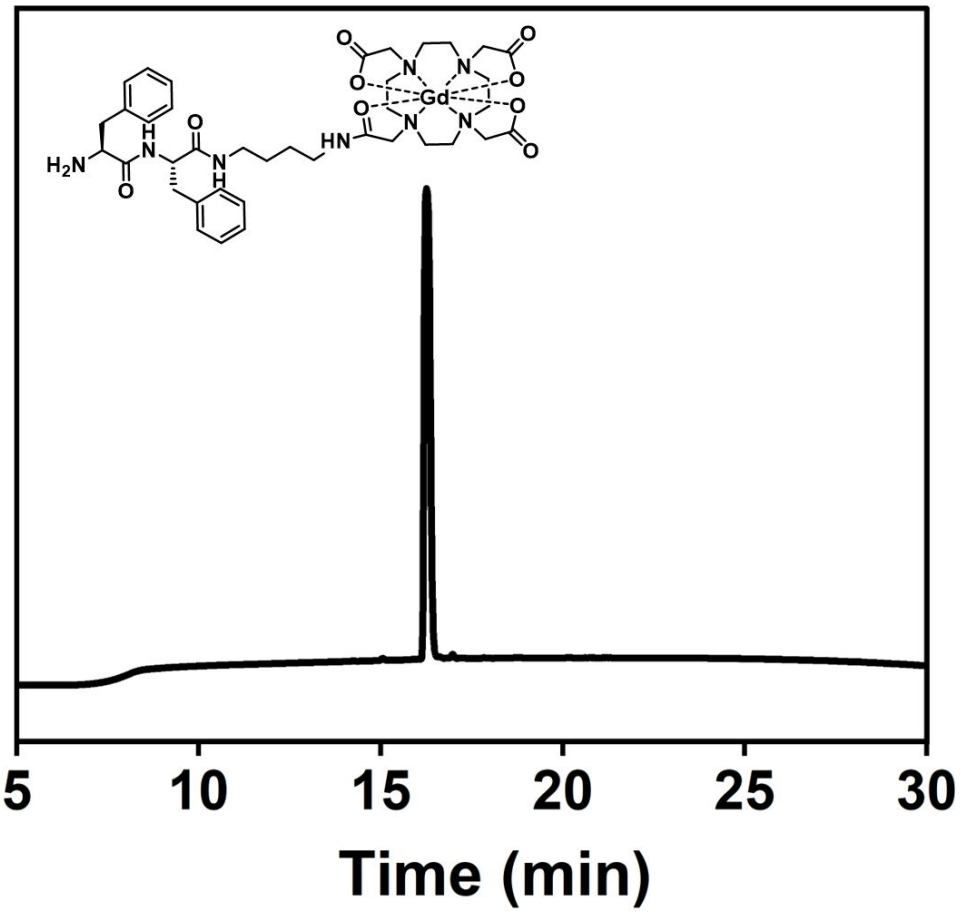


Figure S39. HPLC traces of compound **6b** (FF-C_4-Gd)

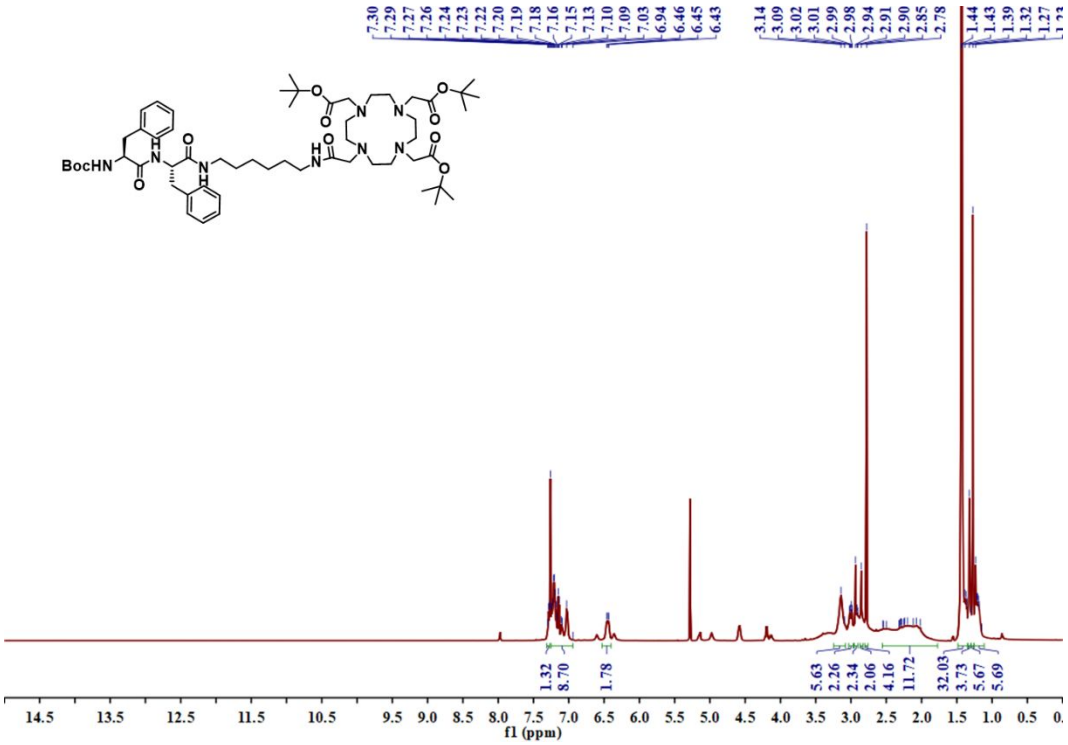


Figure S40. ^{1}H-NMR spectrum of compound **5c** in $CDCl_3$ at room temperature (500 MHz)

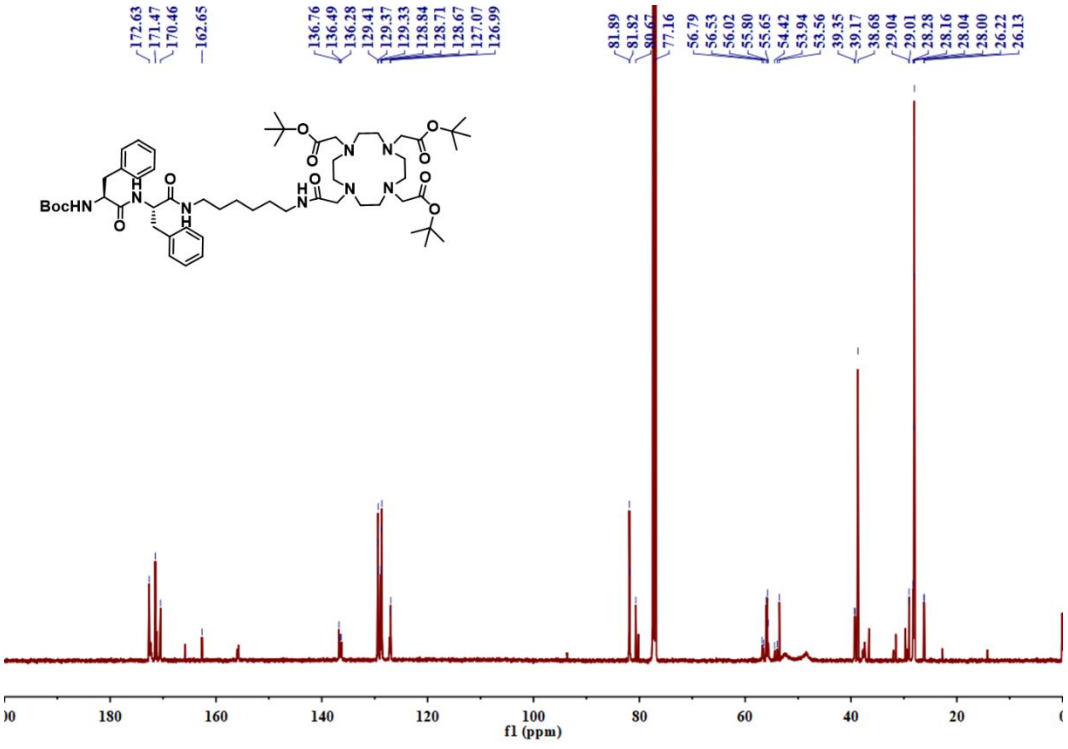


Figure S41. ^{13}C-NMR spectrum of compound **5c** in $CDCl_3$ at room temperature (126 MHz)

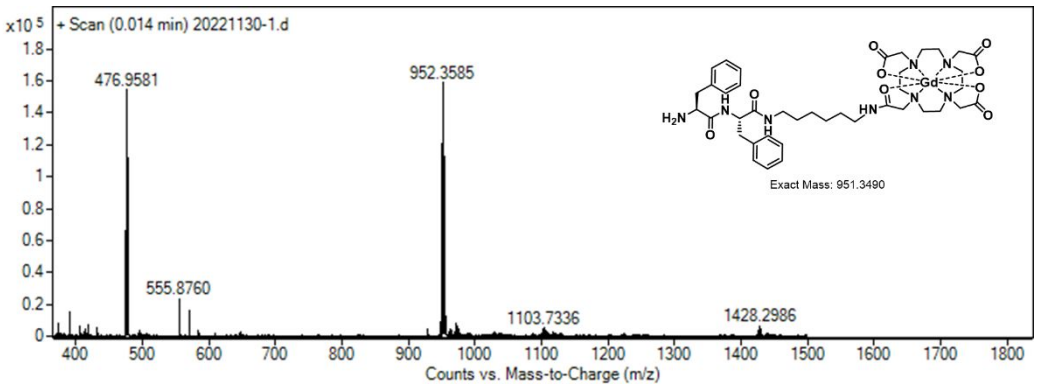


Figure S42. HRMS spectrum of compound **6c** (FF-C_6-Gd)

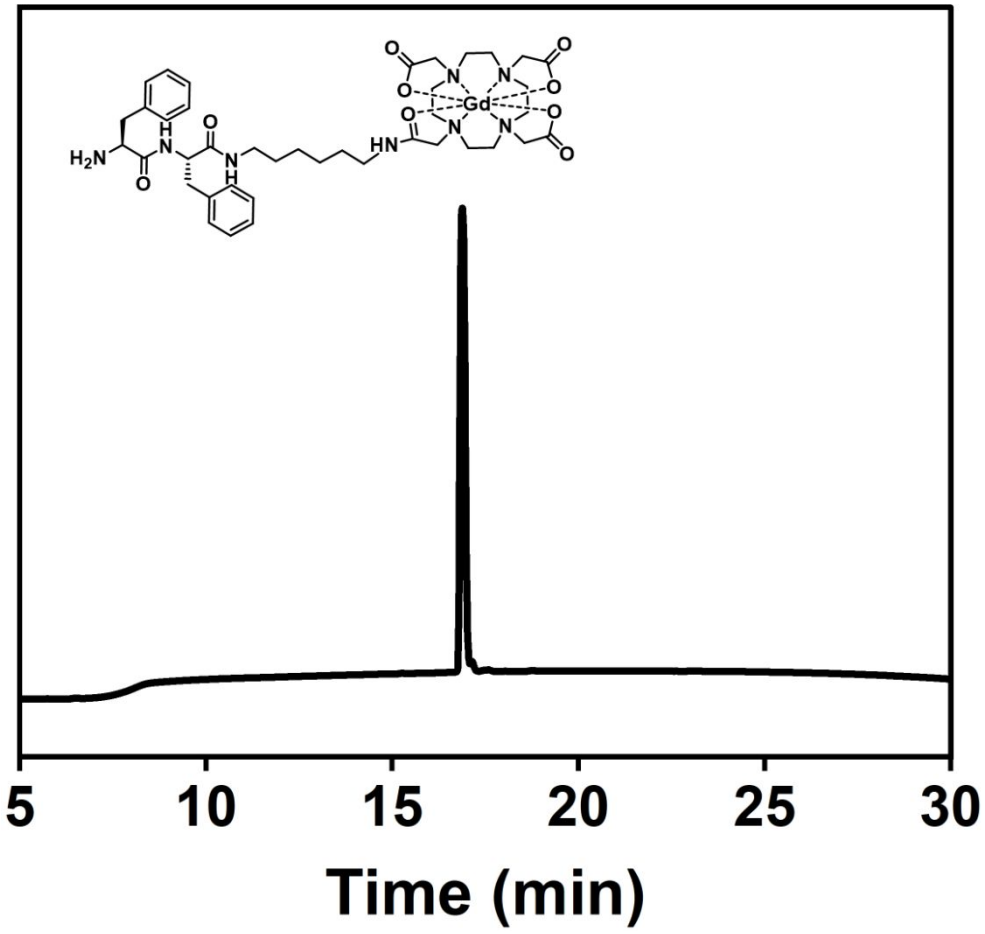


Figure S43. HPLC traces of compound **6c** (FF-C_6-Gd)

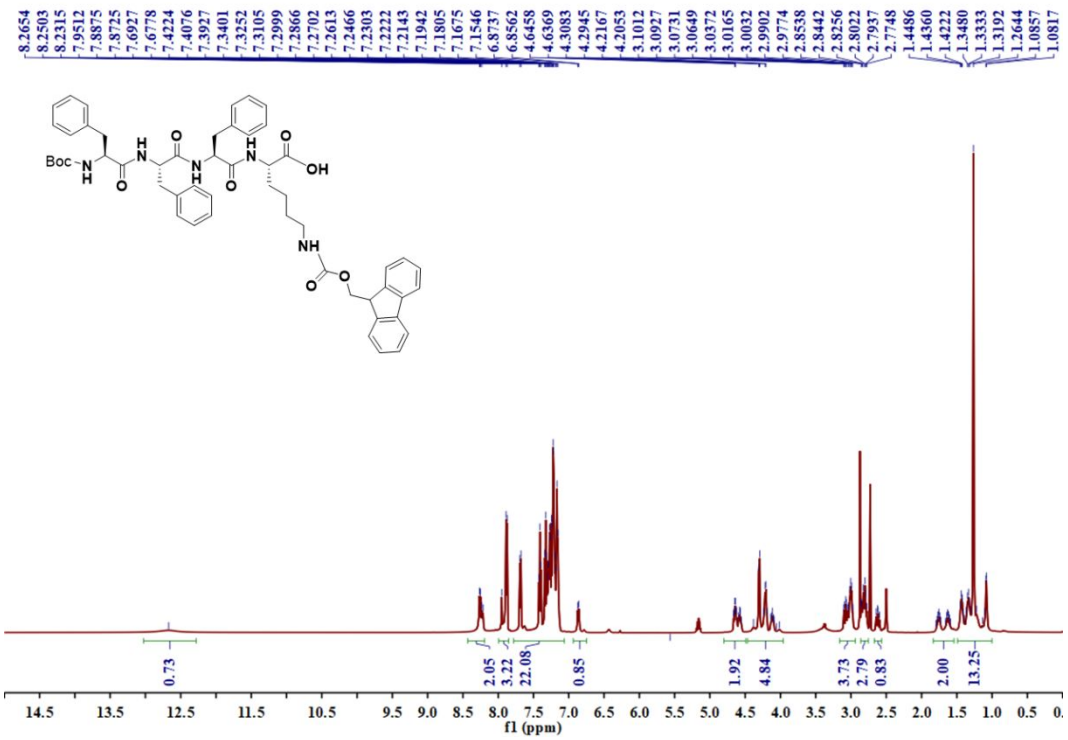


Figure S44. [1]H-NMR spectrum of compound **3** (Boc-FFFK(Fmoc)-OH) in DMSO-*d*6 at room temperature (500 MHz)

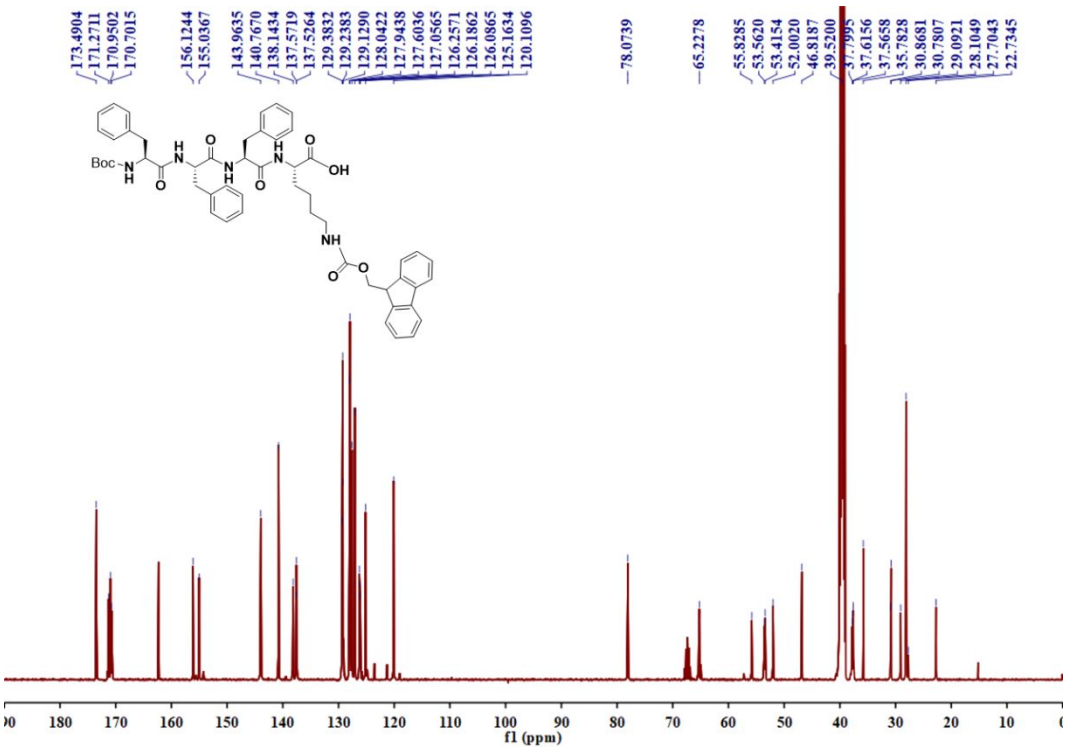


Figure S45. [13]C-NMR spectrum of compound **3** (Boc-FFFK(Fmoc)-OH) in DMSO-*d*6 at room temperature (126 MHz)

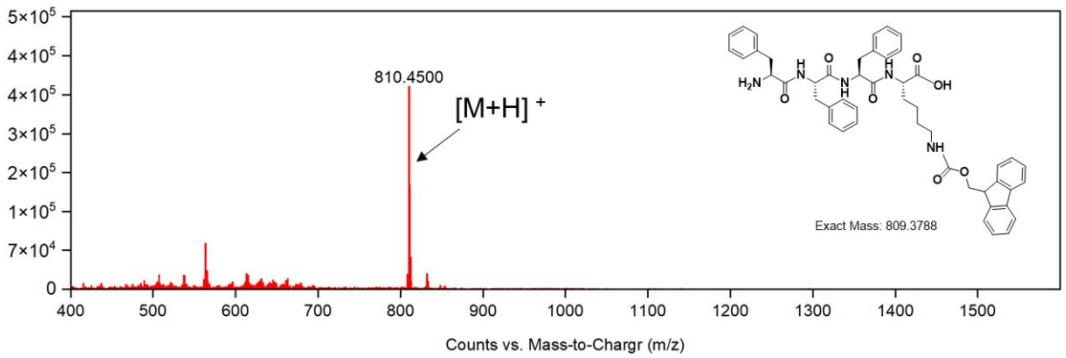


Figure S46. HRMS spectrum of compound **3** (Boc-FFFK(Fmoc)-OH) without protect group.

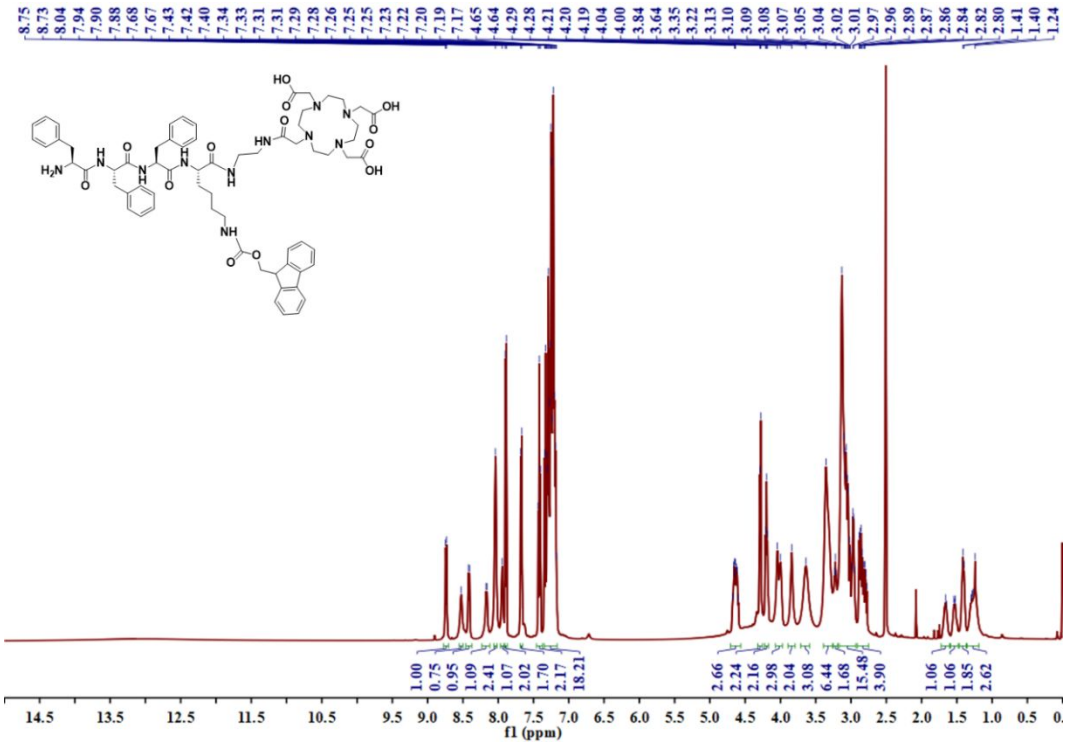


Figure S47. ^{1}H-NMR spectrum of compound **4** (FFFK(Fmoc)-DO3A) in DMSO-*d*6. at room temperature (500 MHz)

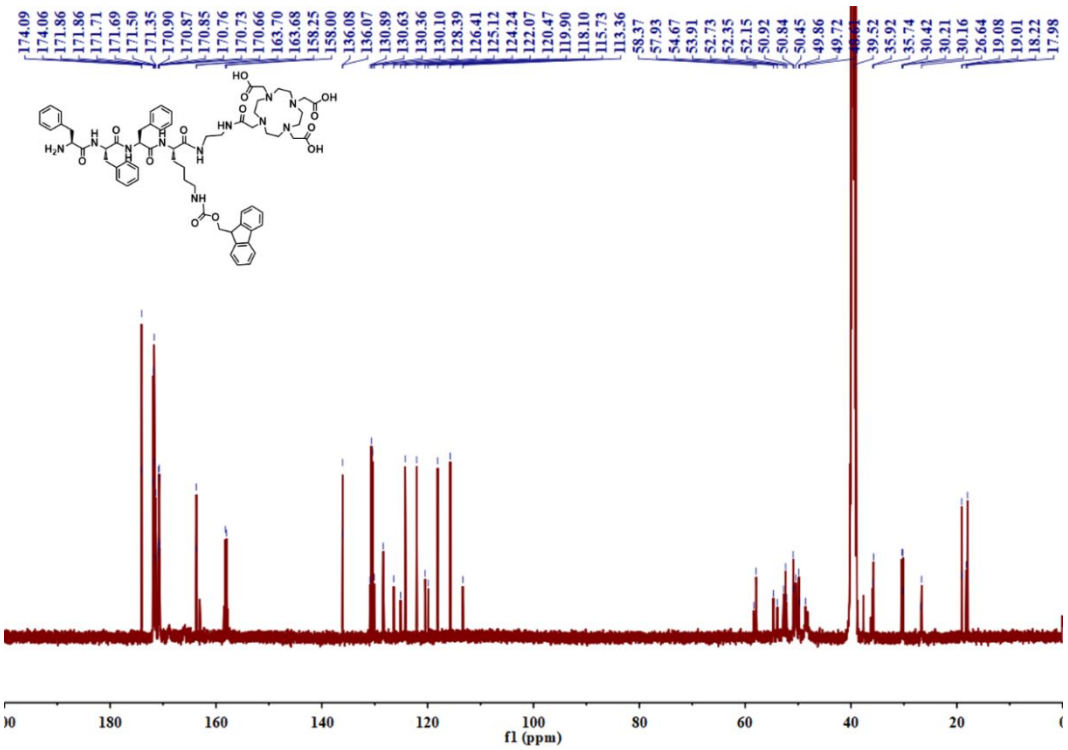


Figure S48. ^{13}C-NMR spectrum of compound **4** (FFFK(Fmoc)-DO3A) in DMSO-*d*6 at room temperature (126 MHz)

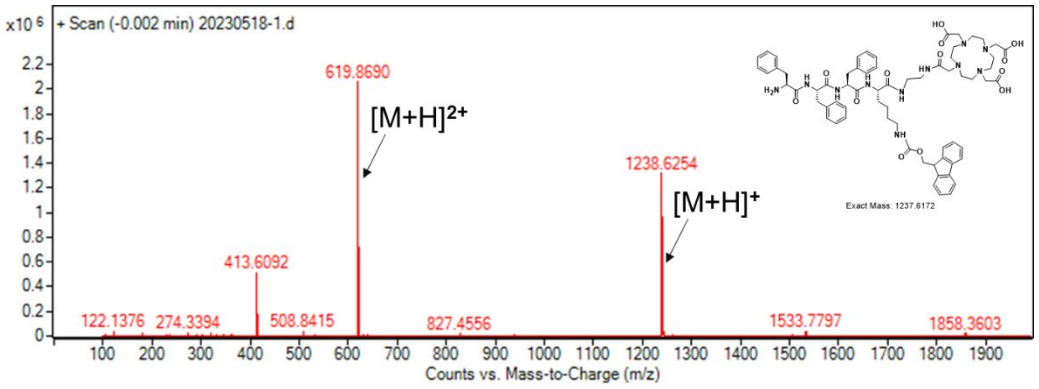


Figure S49. HRMS spectrum of compound **4** (FFFK(Fmoc)-DO3A)

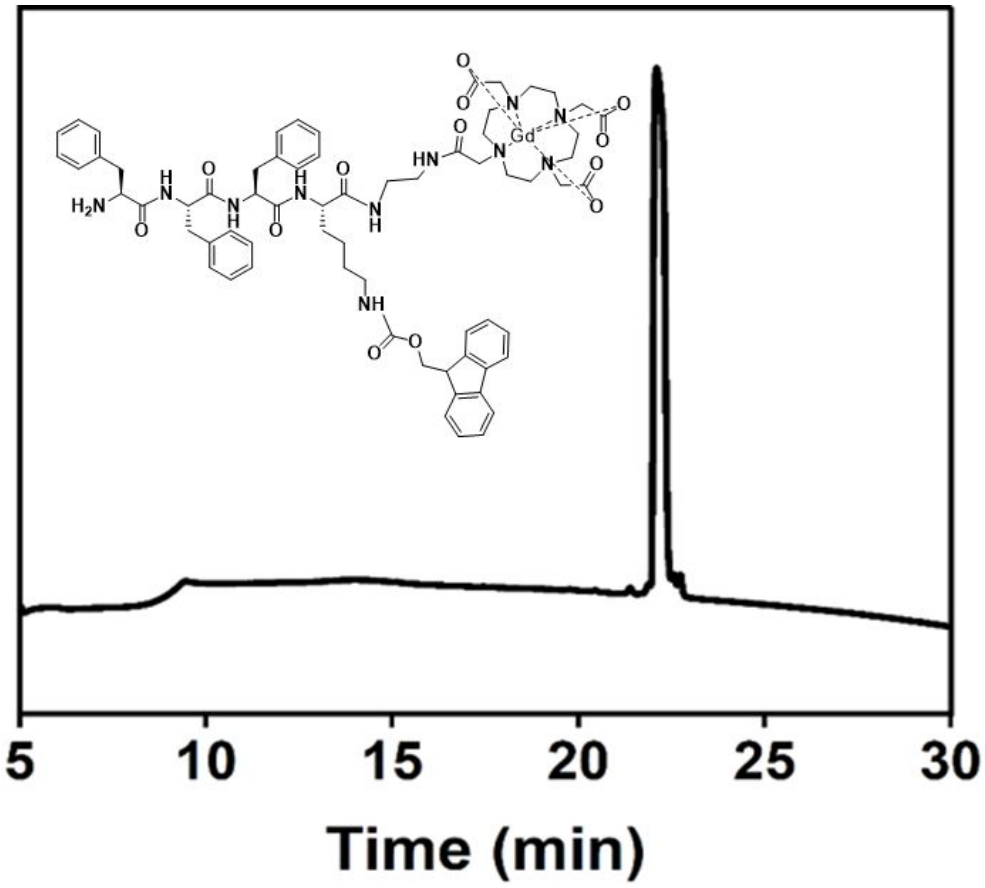


Figure S50. HPLC traces of compound **FFFK(Fmoc)-C_2-Gd (5)**

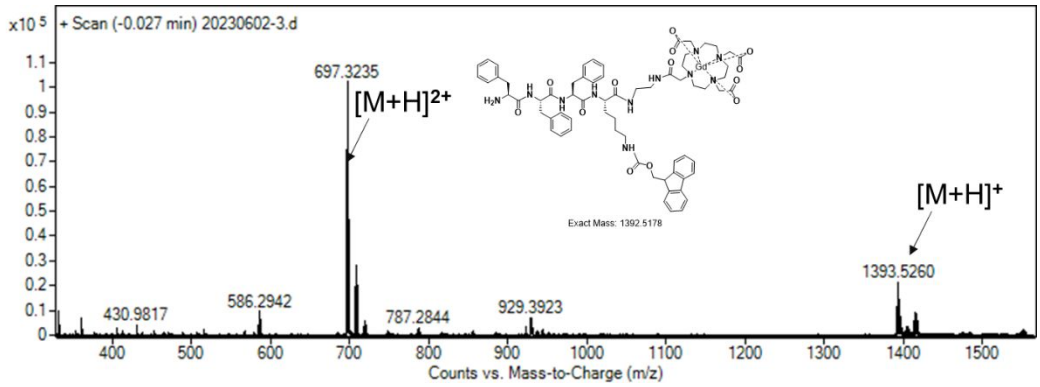


Figure S51. HRMS spectrum of compound **FFFK(Fmoc)-C_2-Gd (5)**

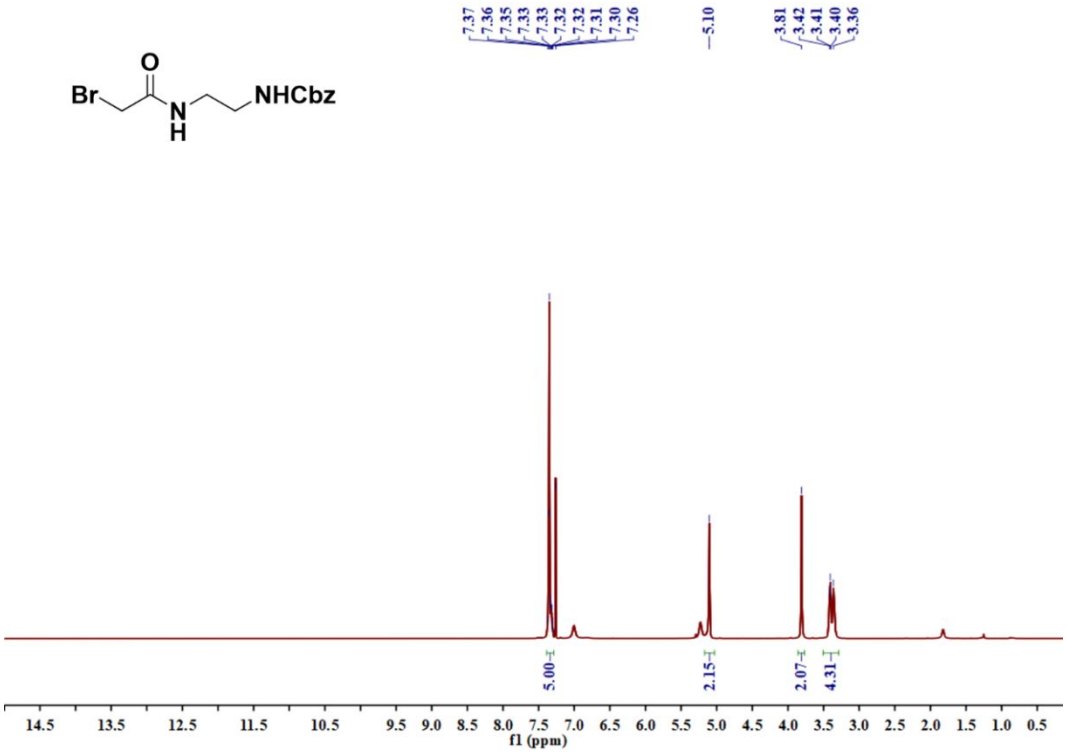


Figure S52. ^{1}H-NMR spectrum of compound **a** in $CDCl_3$ at room temperature (500 MHz)

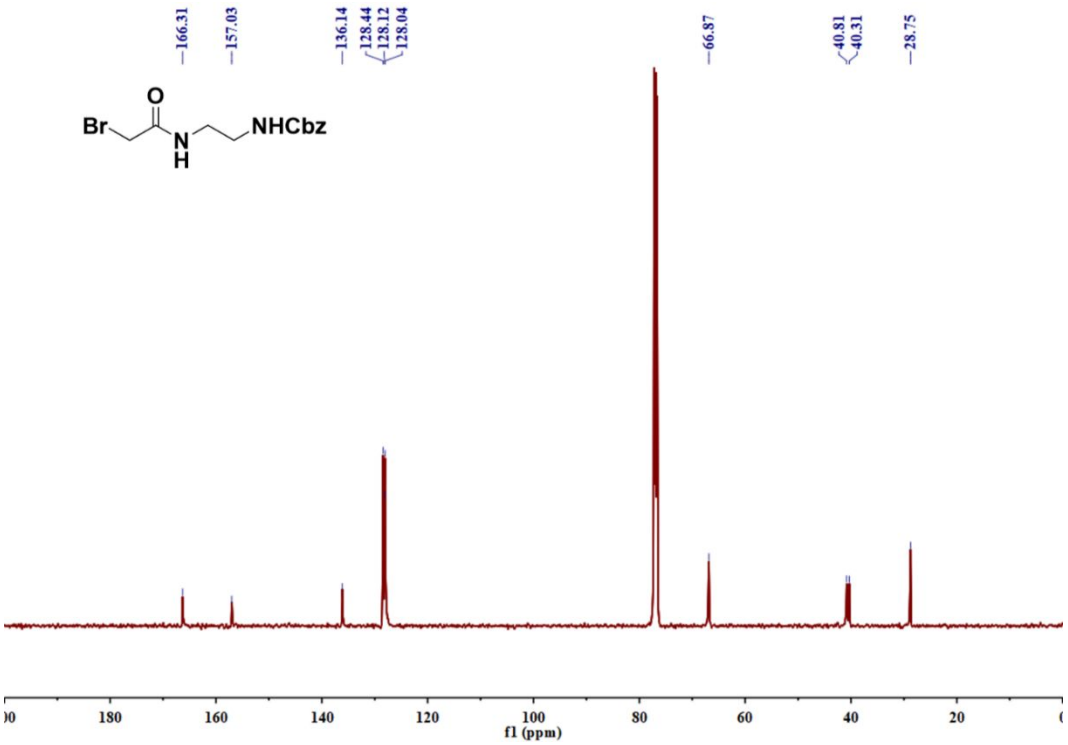


Figure S53. ^{13}C-NMR spectrum of compound **a** in $CDCl_3$ at room temperature (126 MHz)

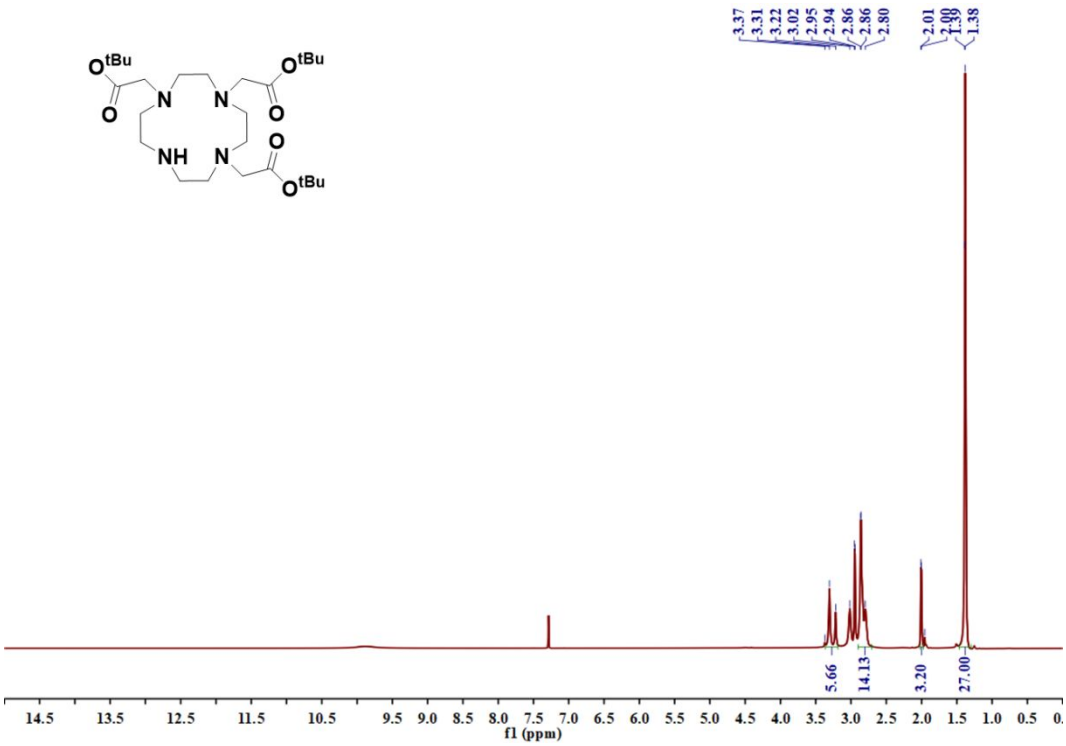


Figure S54. ^{1}H-NMR spectrum of compound **b** in $CDCl_3$ at room temperature (500MHz)

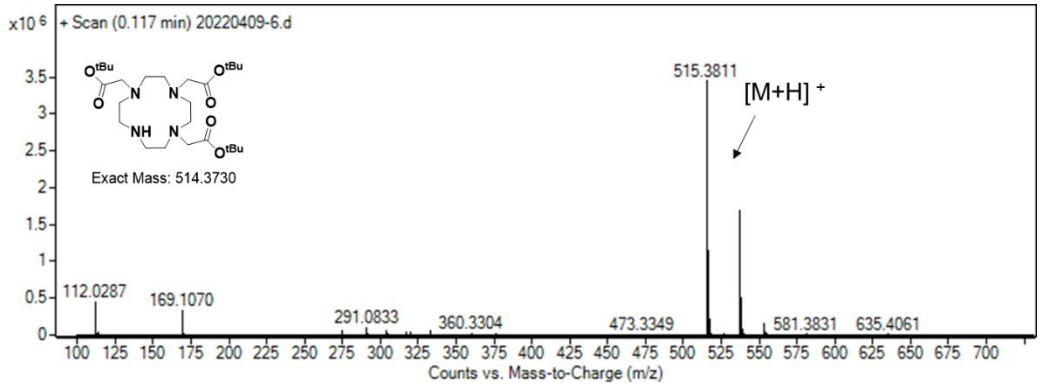


Figure S55. HRMS spectrum of compound **b**

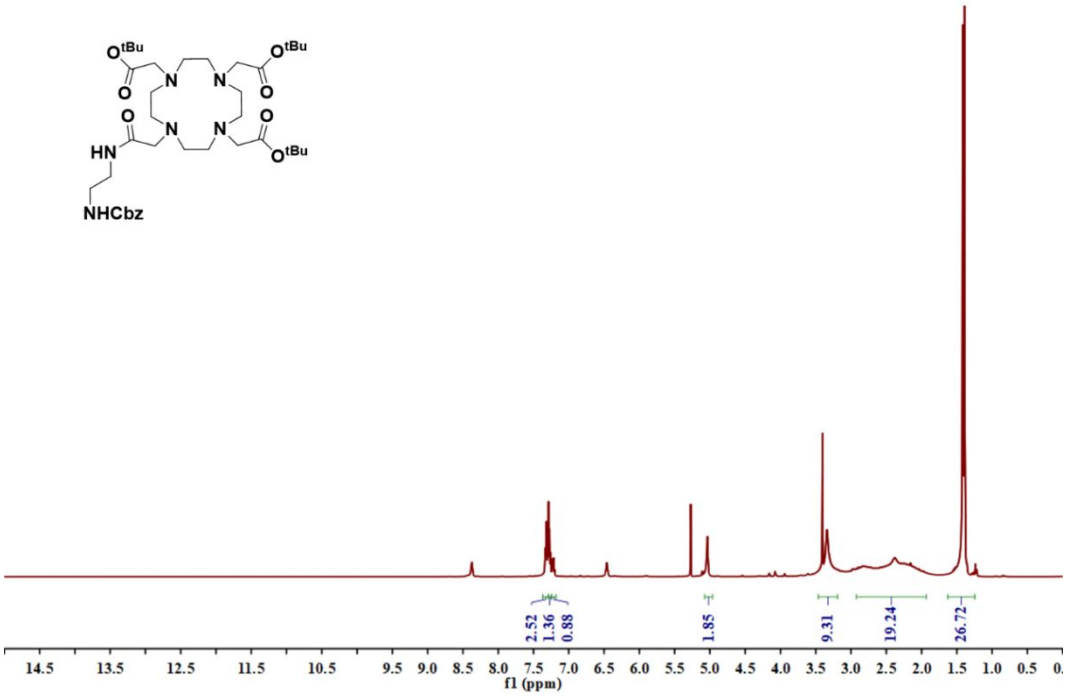


Figure S56. ^{1}H-NMR spectrum of compound **c** in $CDCl_3$ at room temperature (500 MHz)

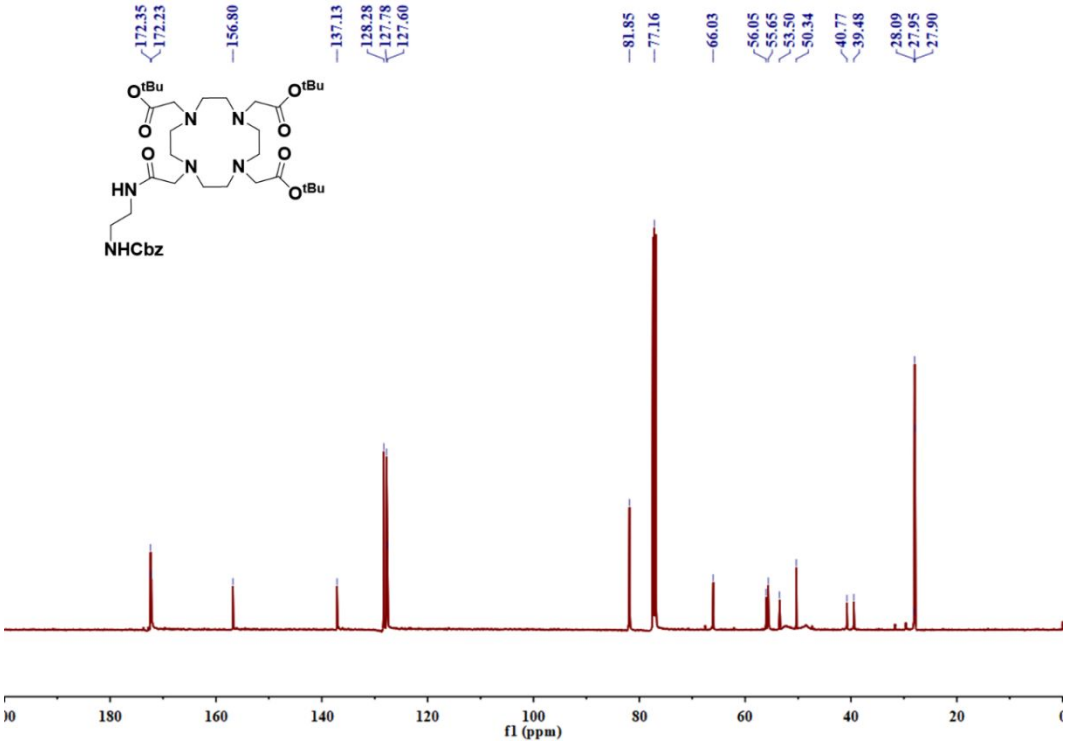


Figure S57. ^{13}C-NMR spectrum of compound **c** in $CDCl_3$ at room temperature (126 MHz)

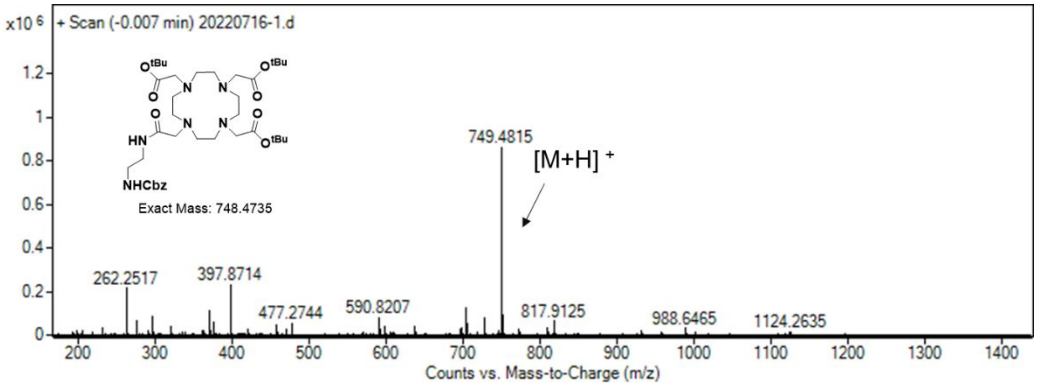


Figure S58. HRMS spectrum of compound **c**

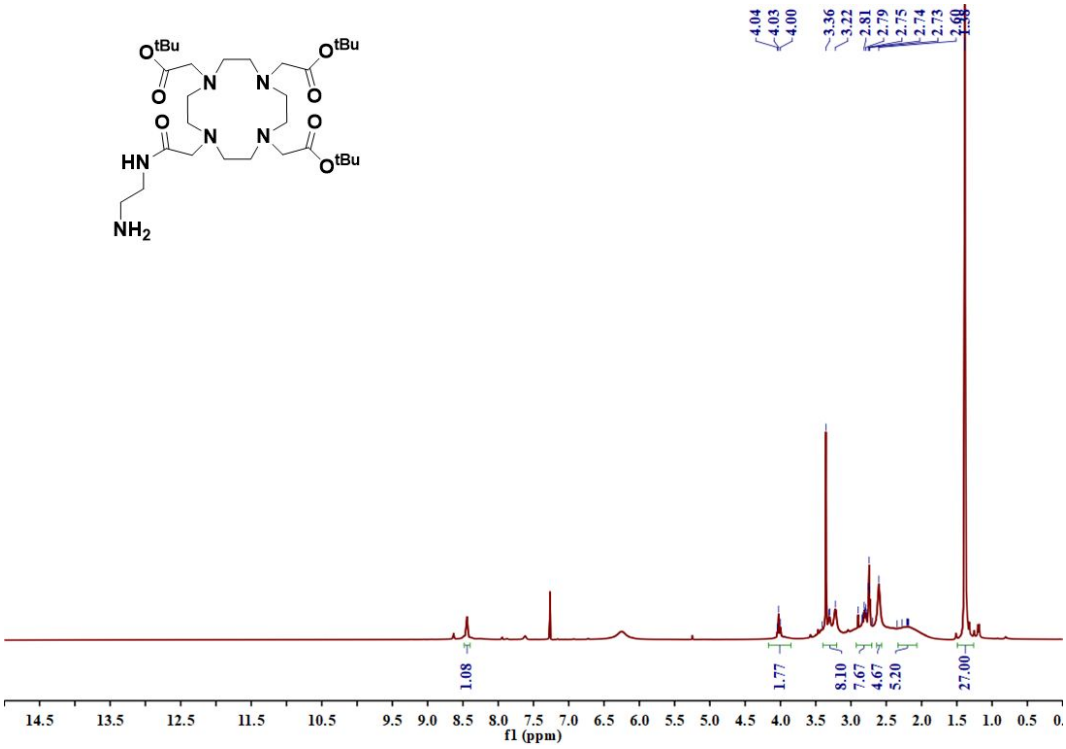


Figure S59. ^{1}H-NMR spectrum of compound **d** in $CDCl_3$ at room temperature (500 MHz)

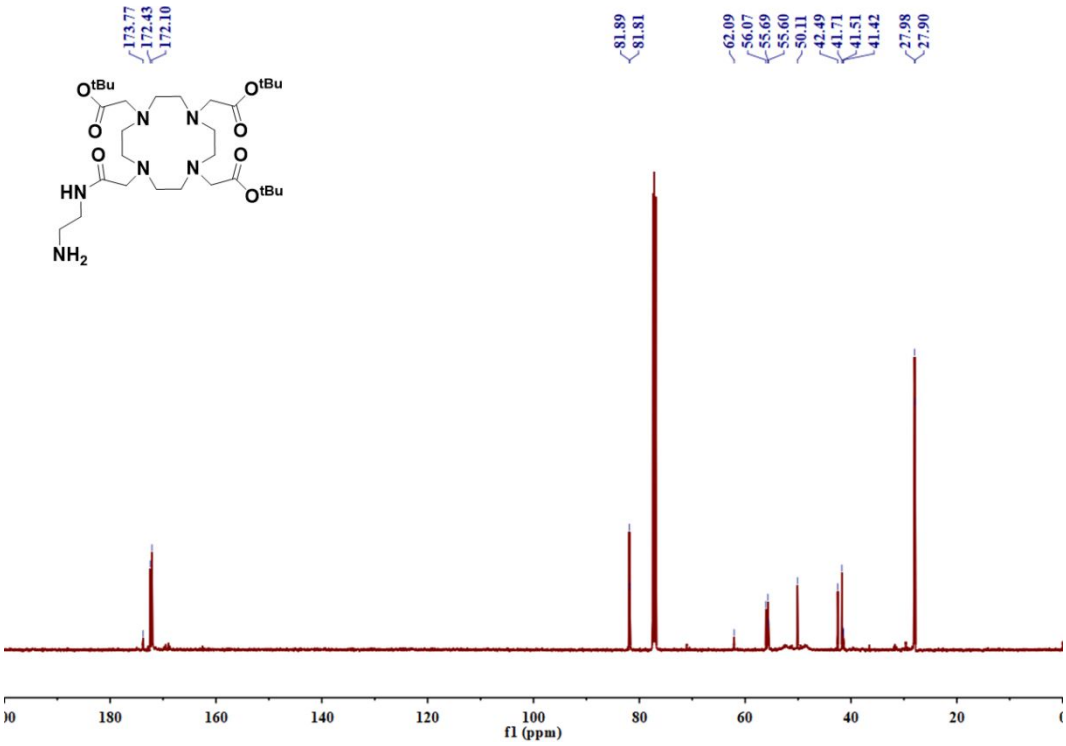


Figure S60. ^{13}C-NMR spectrum of compound **d** in $CDCl_3$ at room temperature (126 MHz)

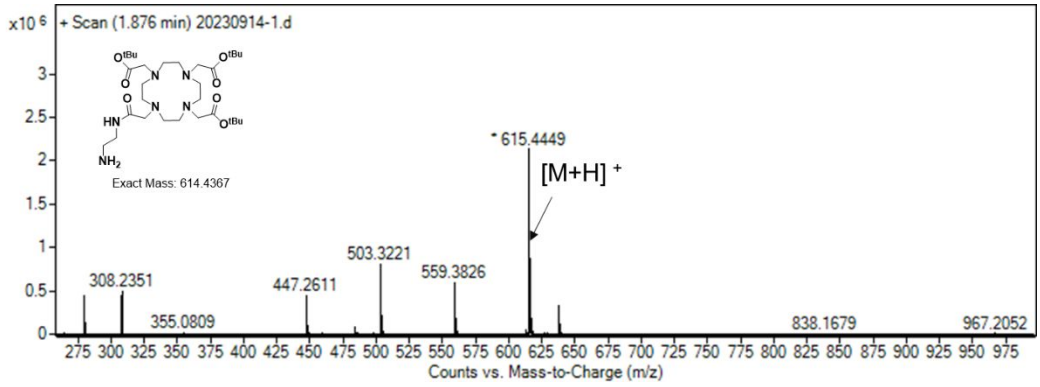


Figure S61. HRMS spectrum of compound **d**

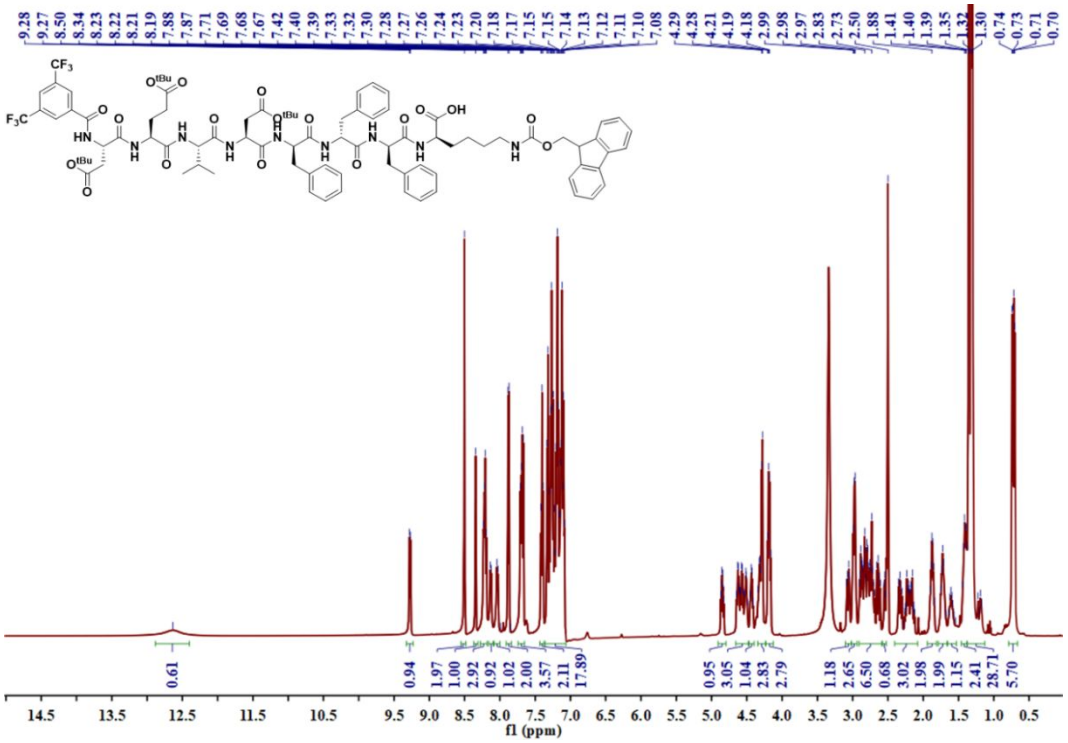


Figure S62. ^{1}H-NMR spectrum of compound **3** ($CF_3D^{OtBu}E^{OtBu}VD^{OtBu}FFFK$(Fmoc)-OH) in DMSO-*d*6 at room temperature (500 MHz)

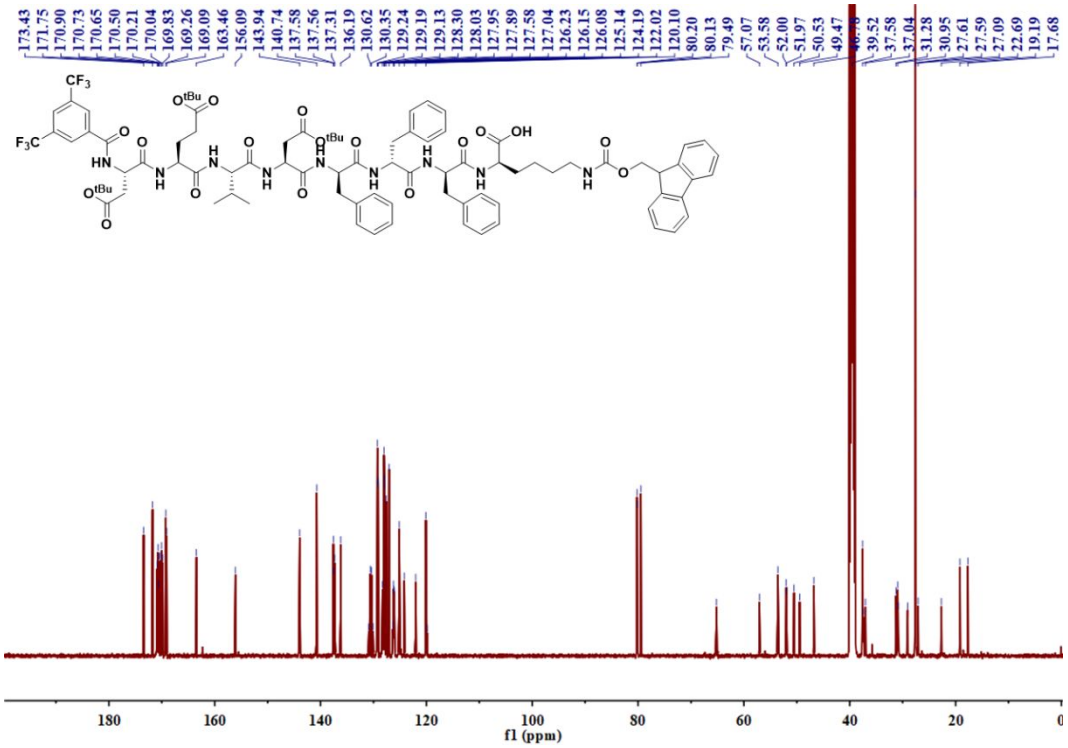


Figure S63. ^{13}C-NMR spectrum of compound **3** ($CF_3D^{OtBu}E^{OtBu}VD^{OtBu}FFFK$(Fmoc)-OH) in DMSO-*d*6 at room temperature (126 MHz)

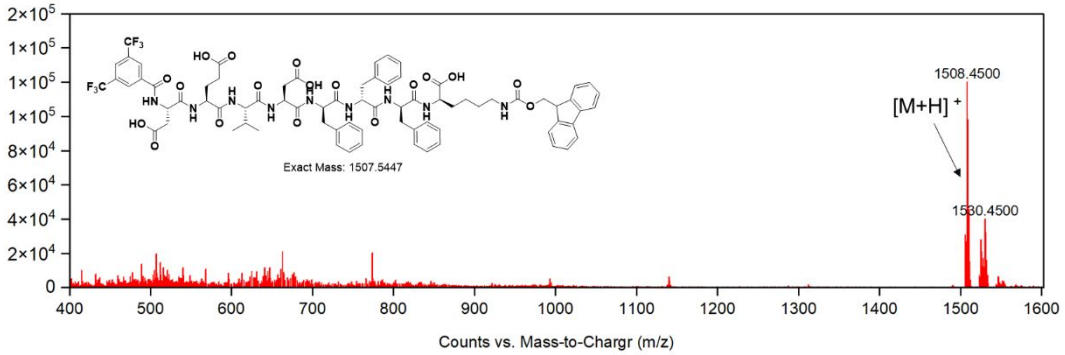


Figure S64. HRMS spectrum of compound **3** without protect group.

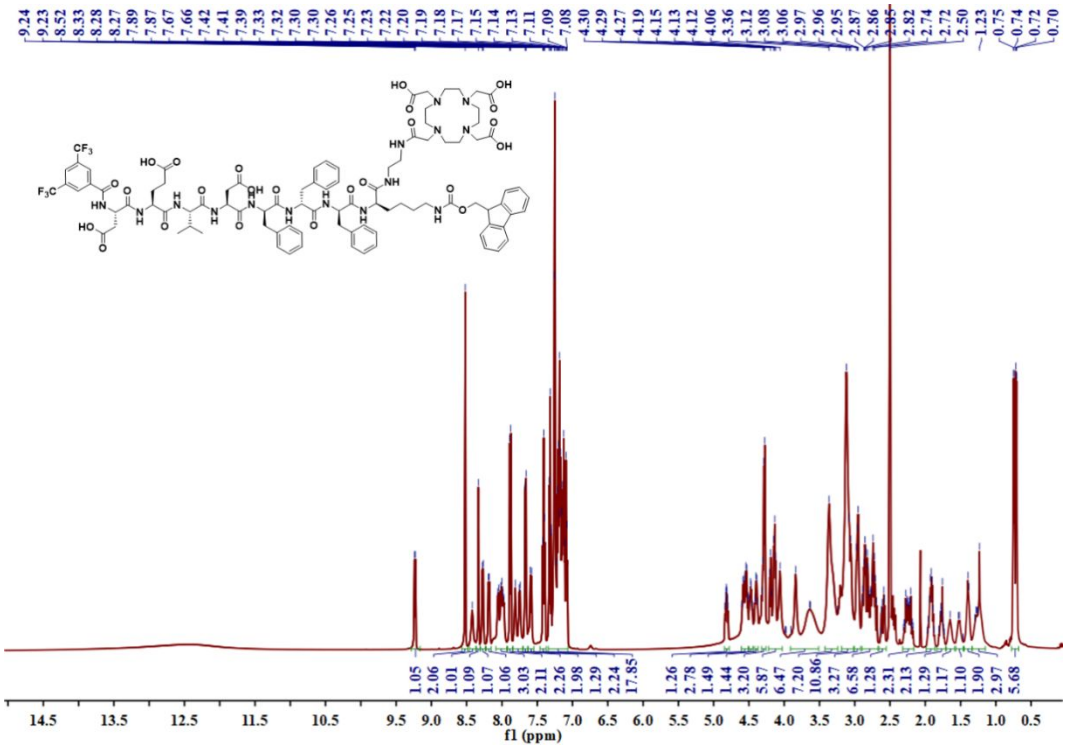


Figure S65. ^{1}H-NMR spectrum of compound **4** (CF_3DEVDFFFK(Fmoc)-DO3A) in DMSO-*d*6 at room temperature (500 MHz)

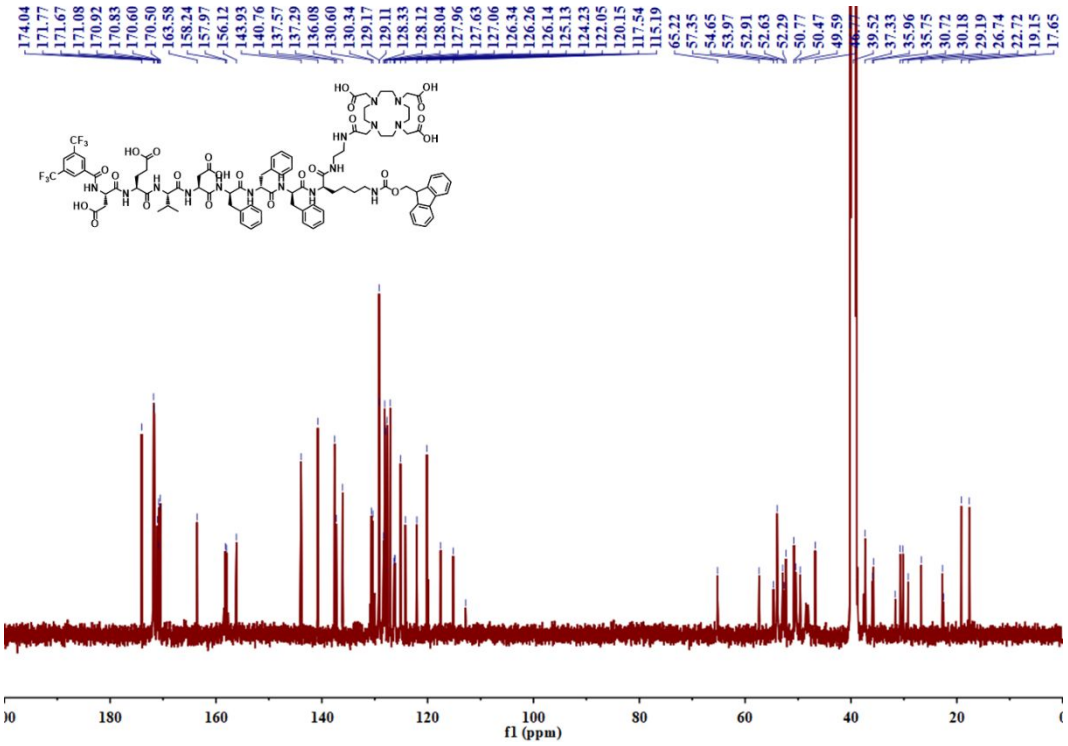


Figure S66. ^{13}C-NMR spectrum of compound **4** (CF_3DEVDFFFK(Fmoc)-DO3A) in DMSO-*d*6 at room temperature (126 MHz)

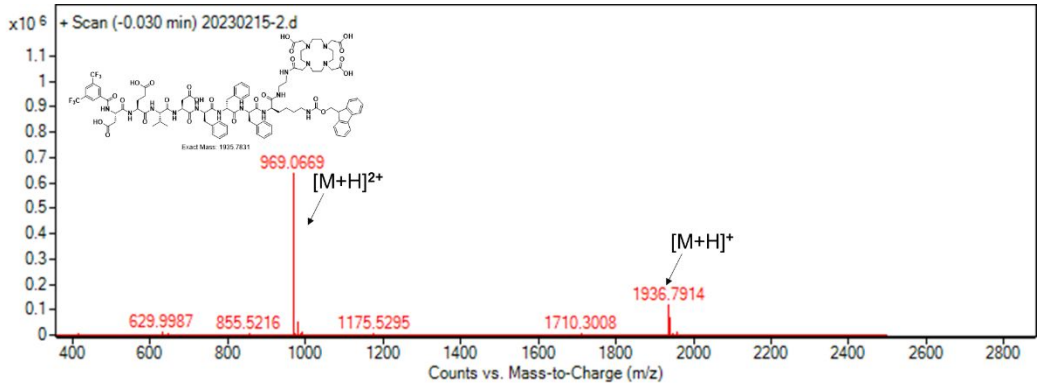


Figure S67. HRMS spectrum of compound **4** (CF_3DEVDFFFK(Fmoc)-DO3A)

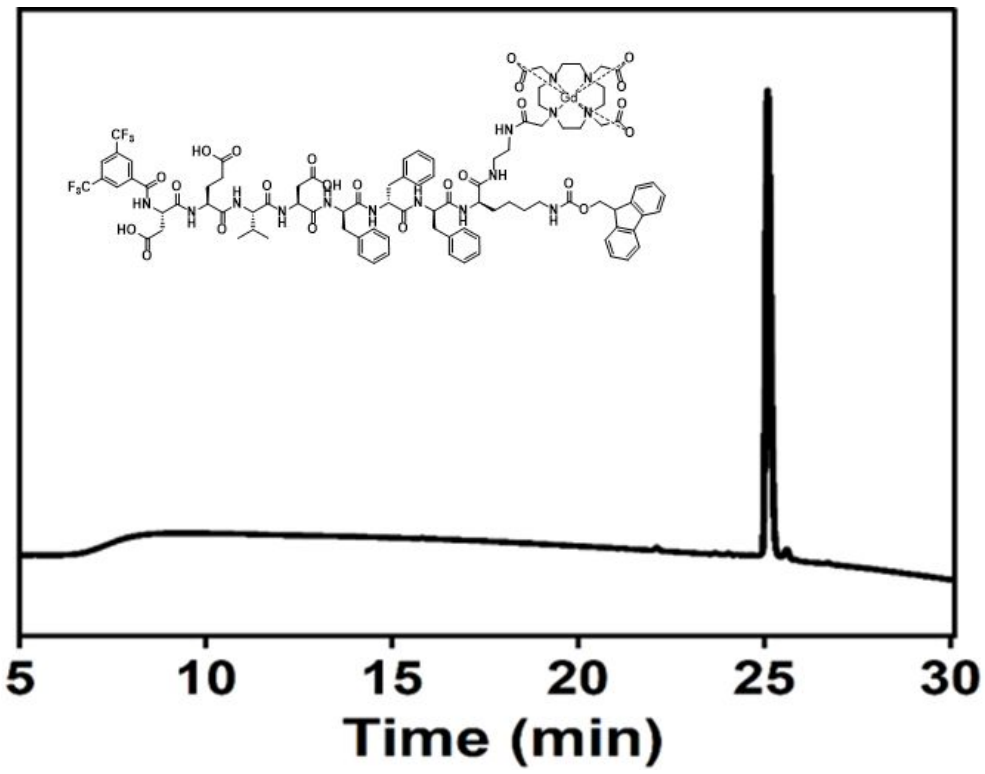


Figure S68. HPLC traces of compound **5** (CF_3DEVDFFFK(Fmoc)-Gd)

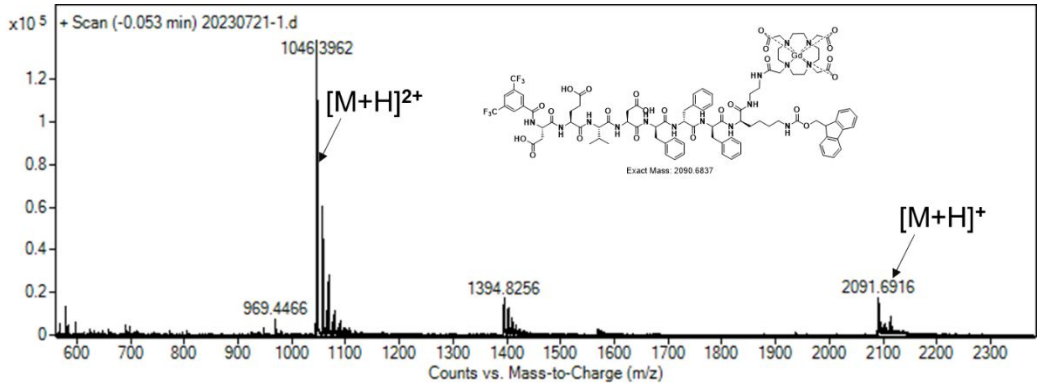


Figure S69. HRMS spectrum of compound **CF_3DEVDFFFK(Fmoc)-Gd (5).**

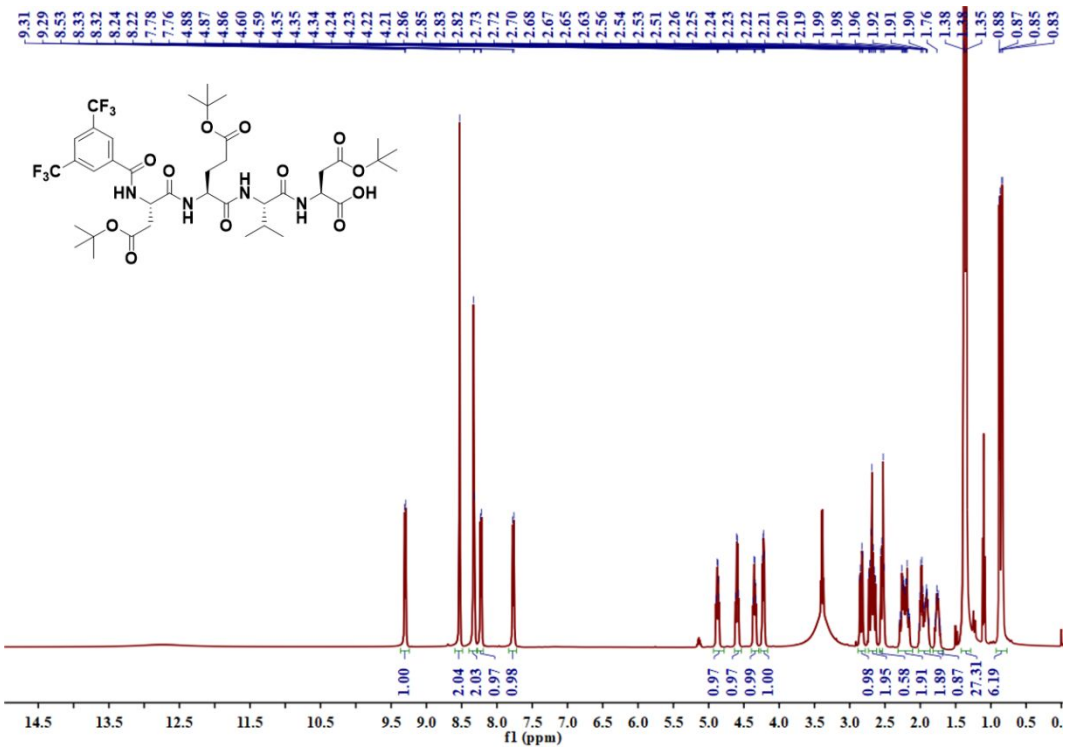


Figure S70. ^{1}H-NMR spectrum of compound **2** ($CF_3D^{OtBu}E^{OtBu}VD^{OtBu}$-OH) in DMSO-*d*6 at room temperature (500 MHz)

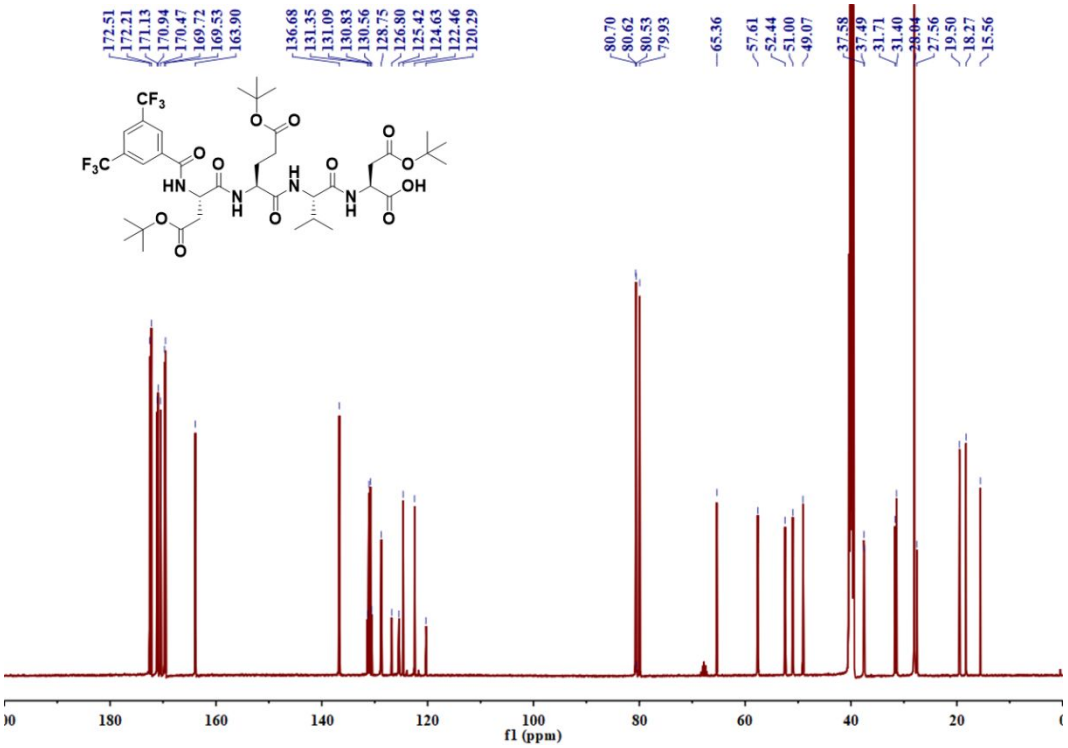


Figure S71. ^{13}C-NMR spectrum of compound **2** ($CF_3D^{OtBu}E^{OtBu}VD^{OtBu}$-OH) in DMSO-*d*6 at room temperature (126 MHz)

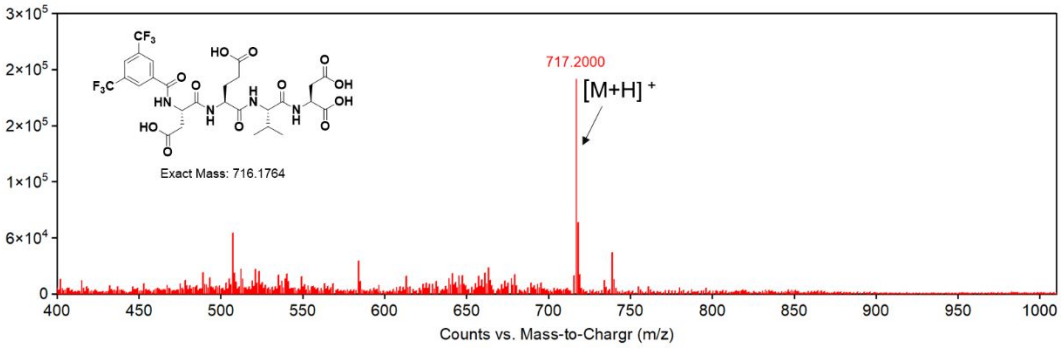


Figure S72. HRMS spectrum of compound **2** (CF_3DEVD-OH).

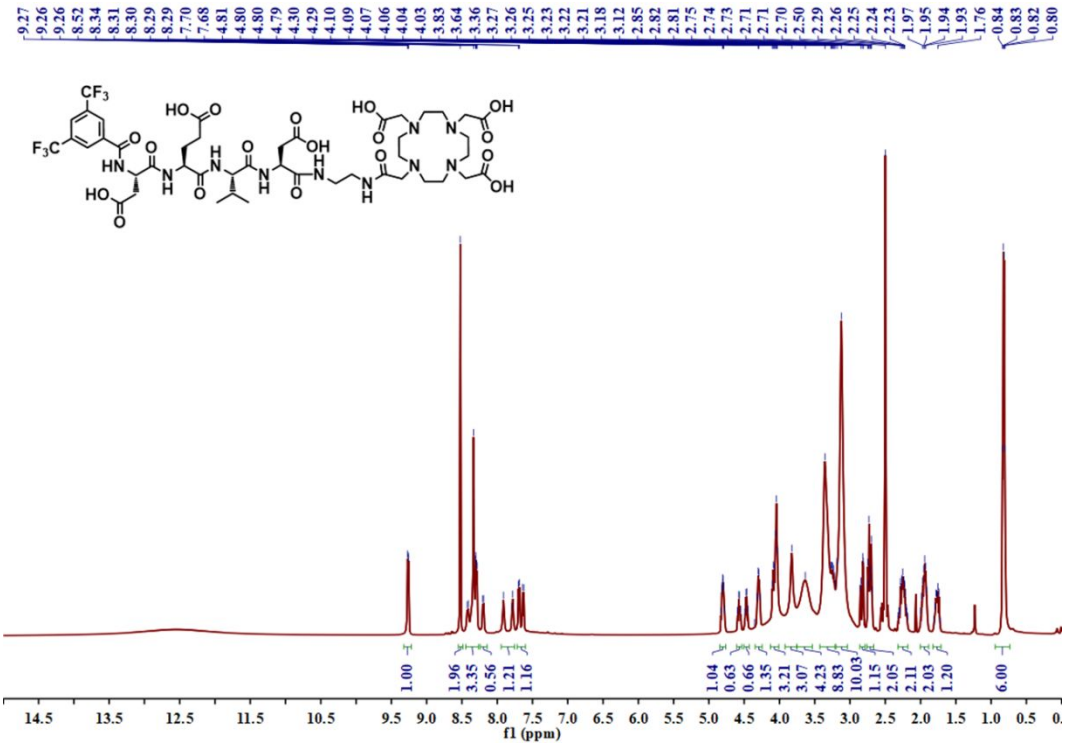


Figure S73. ^{1}H-NMR spectrum of compound **3** CF_3DEVDC_2-DO3A in DMSO-*d*6 at room temperature (500 MHz)

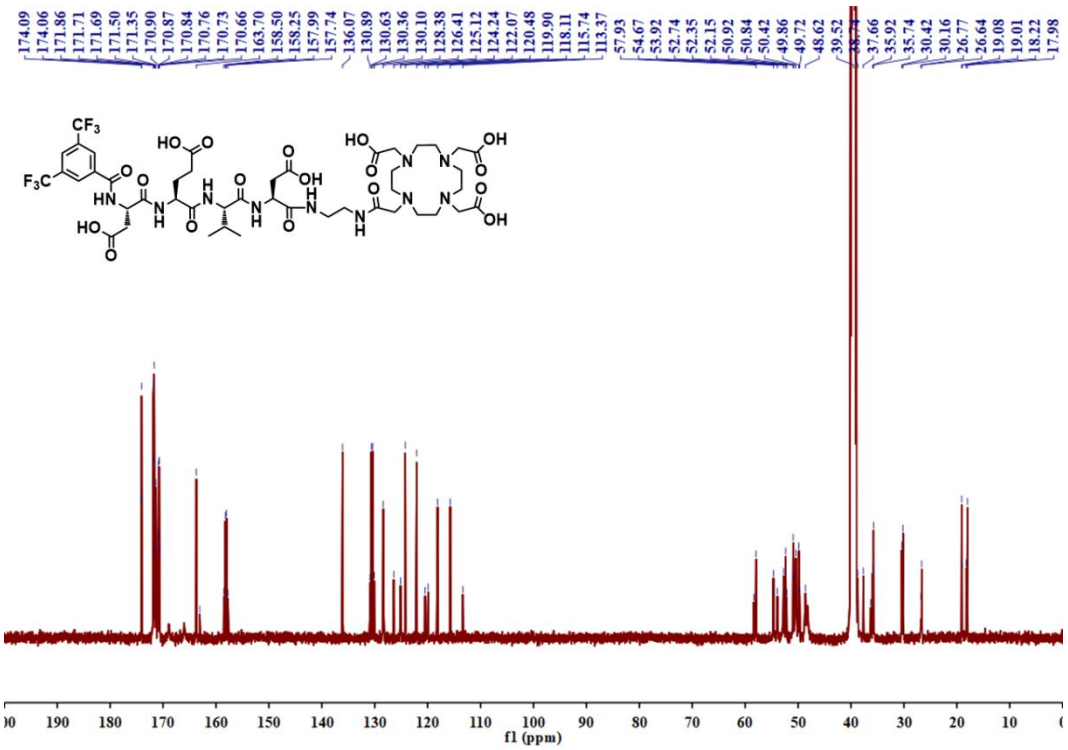


Figure S74. ^{13}C-NMR spectrum of compound **3** CF_3DEVDC_2-DO3A in DMSO-*d*6 at room temperature (126 MHz)

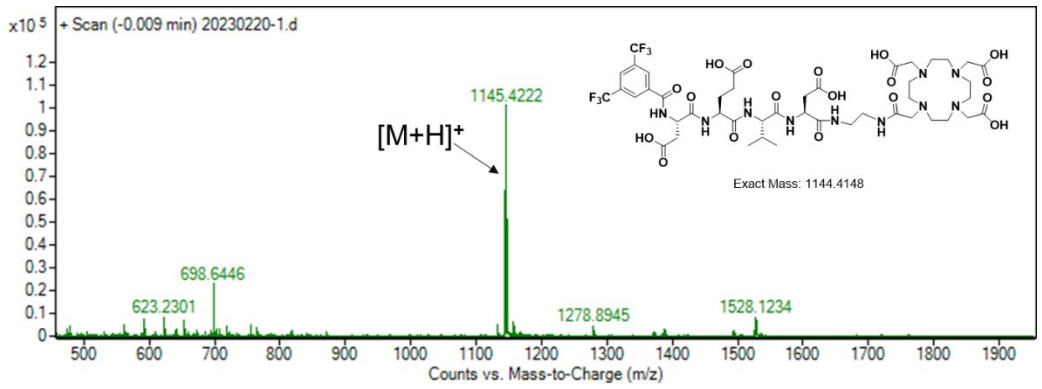


Figure S75. HRMS spectrum of compound **CF_3DEVDC_2-DO3A (3)**

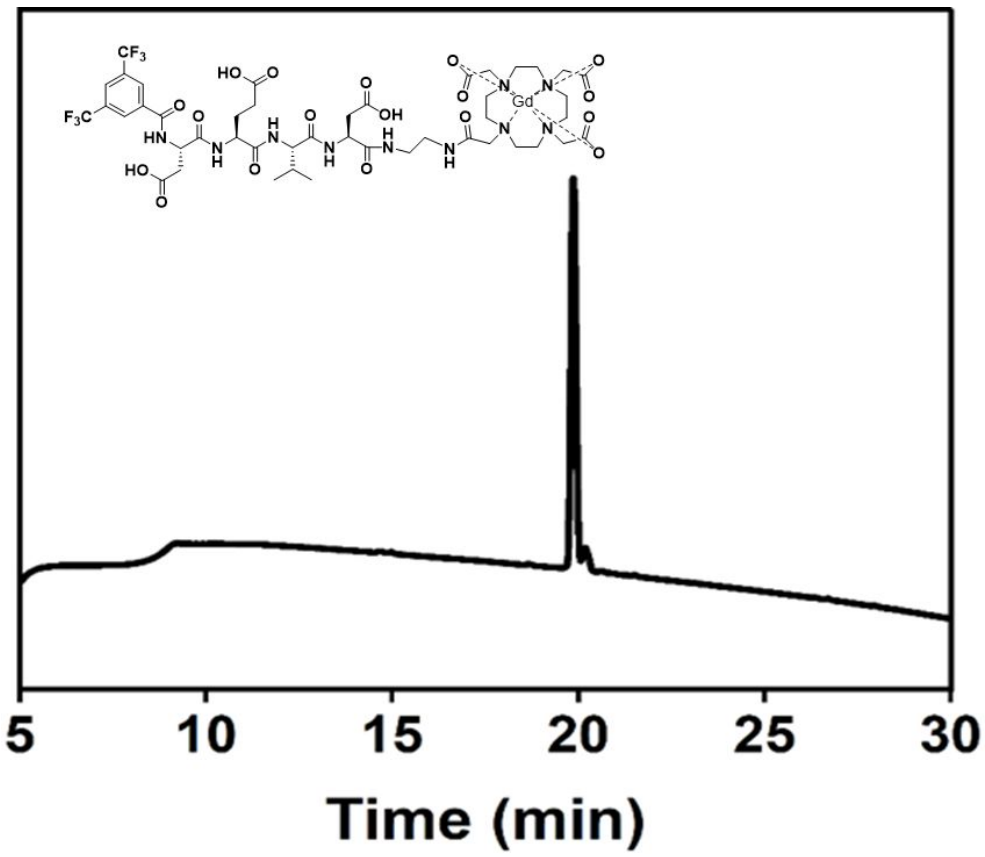


Figure S76. HPLC trace of **CF_3DEVDC_2-Gd (4)**

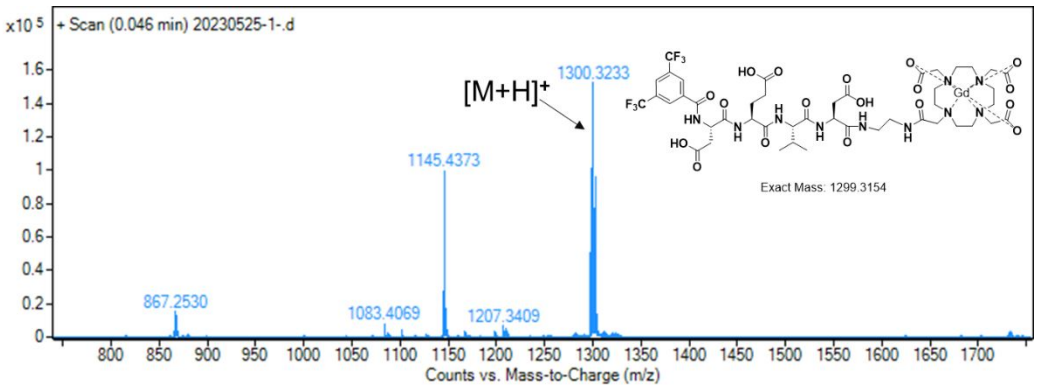


Figure S77. HRMS spectrum of compound **CF_3DEVDC_2-Gd (4)**