Electronic Supplementary Information

A fluorinated bihydrazide conjugate for activatable sensing and imaging of hypochlorous acid by ^{19}F NMR/MRI

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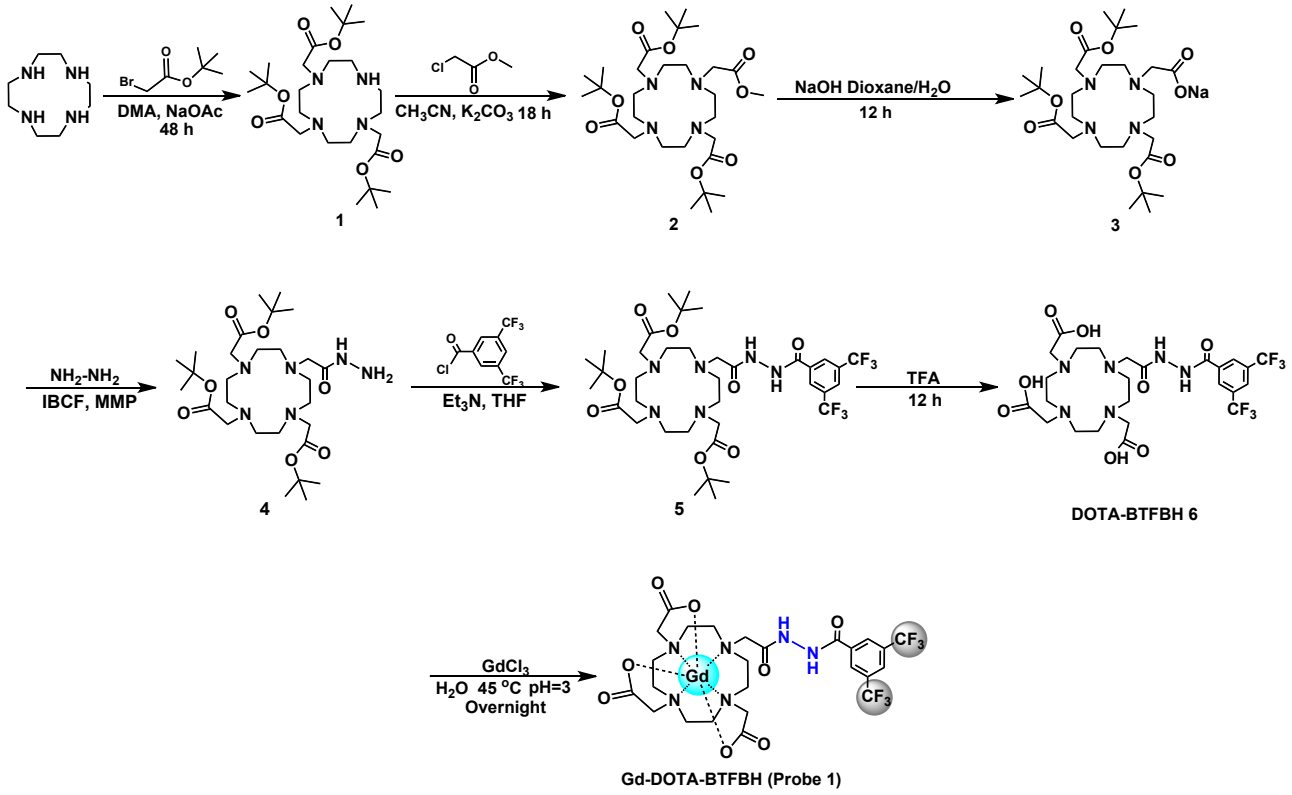
Materials

Cyclen (98%), tert-Butyl bromoacetate(99%), Hydrazine hydrate(99%), 3,5-bis(trifluoromethyl) benzoyl chloride(97%), Gadolinium(III) chloride hexahydrate were purchased from Aladdin (China); Methyl chloroacetate, isobutyl chloroformate 4-methylmorpholine and trifluoroacetic acid were purchased from Inno-Chem (China).Triethylamine (99%) were purchased from J&K Scientific (China). All chemicals were used as received.

Synthesis



Scheme S1. Synthesis of Gd-DOTA-BTFBH (Probe **1**).

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

tert-Butyl bromoacetate (1.3 g, 7.6 mmol, 3.3 equiv) dissolved in 10.0 mL of anhydrous chloroform was added dropwise over 0.5 h to a solution of 1,4,7,10-tetraazacyclododecane (cyclen) (400 mg, 2.32 mmol) and triethylamine (2.3 g, 23.2 mmol, 10.0 equiv) in 40 mL of anhydrous chloroform. The reaction mixture

was stirred for another 2 h and anhydrous K_2CO_3 (0.16 g, 1.16 mmol, 0.5 equiv) was added. After 24 h, the resulting solution was washed with water (3 × 40 mL). The organic phase was dried with $MgSO_4$ and concentrated. This crude product was purified by flash chromatography (15% v/v CH_3OH/CH_2Cl_2) on silica gel to give **1** (3.0 g, 5.8 mmol, 77%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.34 (4 H, m) 3.26 (2 H, m), 3.05 (4 H, m, 2.89-2.85 (12 H, m), 1.47 (27 H, s), the peak for –N*H*– on the ring could not be observed; ESI-MS (m/z) calcd for $C_{26}H_{51}N_4O_6$ [M + H^+]: 515.4, found: 515.4

Synthesis of Compound 2

To a 100 mL round-bottom flask was added **1** (129.5 mg, 0.25 mmol), K_2CO_3 (82.3 mg, 0.6 mmol, 2 equiv) and 20 mL of anhydrous acetonitrile. Methyl chloroacetate (70 mg, 0.65 mmol, 1.1 equiv) was added to the mixture. The suspension was vigorously stirred at room temperature for 4 h. After filtration, the filtrate was concentrated. The residue was purified by flash chromatography (100% CH_2Cl_2 to 20% v/v CH_3OH/CH_2Cl_2) to give **2** (131.8 mg, 0.22 mmol, 90%) as a yellow oil. ^{1}H NMR (400 MHz, CD_3OD): δ 3.76 (3 H, s) 3.29-3.57 (4 H, m), 2.21-3.40 (20 H, m), 1.52 (27 H, s); ESI-MS (m/z) calcd for $C_{29}H_{54}N_4NaO_8$ [M + Na^+]: 609.4, found: 609.3, which is consistent with the previous report (*Nanoscale*, 2017, **9**, 4516-4523).

Synthesis of Compound 3

To a 50 mL round-bottom flask was added **2** (117.2 mg, 0.2 mmol), and 20 mL of NaOH (0.6 M) in dioxane/H_2O (v/v = 2:1). The mixture was vigorously stirred at 50 ^{o}C overnight and concentrated *in vacuo*. Subsequently, the residue was dissolved in water and extracted with DCM (20 mL × 3). The organic phases were collected, dried with Na_2SO_4, and concentrated to give **3** (107.12 mg, 0.18 mmol, 90%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 3.29-3.57 (4 H, m),2.02-3.22 (m, 20 H), 1.48 (s, 27 H); ESI-MS (m/z) calcd for $C_{28}H_{51}N_4NaO_8$ [M + H^+]:595.4, found: 595.4, which is consistent with the previous report (*Tetrahedron Lett.*, 2009, **50**, 2929-2931).

Synthesis of Compound 4

3 (238 mg, 0.4 mmol) and 15 mL of anhydrous acetonitrile were added to a round-bottom flask under N_2. Then isobutyl chloroformate (0.4 mmol, 50.8 μL, 1.0 equiv) and 4-methylmorpholine (0.4 mmol, 45 μL, 1.0 equiv) were added. The mixture was vigorously stirred at 0 °C for 30 min before warmed to room temperature. Hydrazine hydrate (0.4 mmol, 35 μL) was added and the resulting mixture was further stirred for 2 h then concentrated *in vacuo*. Subsequently, the residue was dissolved in DCM (20 mL) and washed with water (20 mL × 3). The organic phase was dried with $MgSO_4$ and concentrated. The crude product was purified by flash chromatography (20% v/v CH_3OH/CH_2Cl_2) on silica gel to give **4** (165 mg, 0.28 mmol, 70%) as an off-white powder. ^{1}H NMR (600 MHz, CD_3OD): δ1.81-3.84(a set of very broad and multiple peaks with an integration corresponding to 24 H), 1.50 (s, 27 H, t-Bu), the peaks for –$NHNH_2$ could not be observed; ^{13}C NMR (151 MHz, CD_3OD): δ174.04, 173.03, 171.63, 81.45, 55.81, 55.33, 55.38, 55.17, 54.81, 54.30, 53.2-50.9 (m), 27.06. HR-ESI-MS (m/z) calcd for $C_{28}H_{54}N_6NaO_7$ [M + Na^+]:609.3946, found: 609.3936.

Synthesis of Compound 5

4 (117.2 mg, 0.2 mmol) in 15 mL of anhydrous THF was cooled to 0 °C under N_2. Then Et_3N (2 mmol, 278 μL,10 equiv) and 3,5-bis(trifluoromethyl) benzoyl chloride (2 mmol, 363 μL,10 equiv) was added dropwise. The mixture was stirred at 0 °C for 18 h and concentrated *in vacuo*. This crude product was purified by flash chromatography (6% v/v CH_3OH/CH_2Cl_2) on silica gel to give **5** (124 mg, 0.15 mmol, 75%) as a yellow powder. ^{1}H NMR (600 MHz, CD_3OD): δ 8.49 (s 2 H), 8.23 (s, 1 H), δ2.80-4.64(a set of very broad and multiple peaks with an integration corresponding to 24 H), 1.50 (s, 27 H, *t*-Bu), the peaks for –N*H*N*H*– could not be observed; ^{13}C NMR (151 MHz, CD_3OD): δ 164.43, 160.43 (q, *J* C-F = 37.2 Hz), 134.38, 131.95 (q, *J* C-F = 33.2 Hz), 128.05, 125.9-125.6 (m), 125-124.5 (m) 123.01 (q, *J* C-F = 271.8 Hz), 116.19 (q, *J* C-F = 288.4 Hz), 55.2-52.5 (m), 26.95.HR-ESI-MS (m/z) calcd for $C_{37}H_{57}F_6N_6NaO_8$ [M + Na^+]:849.3956, found: 849.3940.

Synthesis of DOTA-BTFBH (6)

5 (124 mg, 0.15 mmol) and 3 mL of anhydrous trifluoroacetic acid was added to a round-bottom flask. The mixture was heated to 50 ^{o}C, stirred for 6 h and concentrated to give crude DOTA-BTFBH (**6**) as a red oil, which was directly used in the next step without further purification. A small portion of the crude DOTA-BTFBH (**6**) was purified by HPLC to give pure DOTA-BTFBH (**6**) as a white solid: ^{1}H NMR (600 MHz, CD_3OD): δ 8.48 (s 2 H), 8.20 (s, 1 H), δ2.95-4.45(a set of very broad and multiple peaks with an integration corresponding to 24 H), the peaks for –N*H*N*H*– and –COO*H* could not be observed; ^{13}C NMR (151 MHz, CD_3OD): δ 164.62, 160.33 (q, *J* C-F = 36.2 Hz), 134.24, 131.77 (q, *J* C-F = 33.2 Hz), 128.11, 125.28, 123.06 (q, *J* C-F = 273.3 Hz), 120.36, 116.02 (q, *J* C-F = 289.9 Hz), 54.2-52.4 (m). HR-ESI-MS (m/z) calcd for $C_{25}H_{33}F_6N_6O_8$ [M + H^+]:659.2258, found: 659.2261.

Synthesis of Gd-DOTA-BTFBH (Probe 1)

DOTA-BTFBH (65 mg, 0.1 mmol) was dissolved in 10 mL of DI H_2O and $GdCl_3$•$6H_2O$ (194.7 mg, 0.3 mmol, 3.0 equiv) was added. The pH was adjusted to ~ 4 with 0.1 M NaOH and the resulting mixture was stirred overnight at 50 ^{o}C. After lyophilization, the obtained while solid was dissolved in CH_3CN and further purified by gradient elution using HPLC (ZORBAX SB-18 column from 98% CH_3CN/2% H_2O to 70% CH_3CN/30% H_2O, 025 min). HR-ESI-MS (m/z) calcd for $C_{25}H_{30}F_6GdN_6O_8$ [M + H^+]: 814.1271, found: 814.1287.

MS Characterization. The molecular weights of the synthesized compounds were measured on an Esquire 3000 Plus electrospray ionization instrument using an ICR analyzer (ESI-MS) and Bruker FT-MS.

^{1}H MRI Characterization. Relaxivity measurements and ^{1}H MRI phantom imaging was performed on a 0.5 T Niumag NMI20-Analyst system (Suzhou Niumag Analytical Instrument Corporation).

^{19}F NMR/MRI Characterization. All ^{19}F NMR experiments were carried out on a Bruker AVANCE III HD Ascend 600 MHz spectrometer using a 5 mm BBFO cryoprobe. All ^{19}F NMR spectra were acquired with 18 μs delay and 200 scans. Samples were prepared with 5% D_2O/H_2O and CF_3COONa (-75.4 ppm) was used as a reference for chemical shift. All *in vivo* ^{1}H/^{19}F MRI and ^{19}F NMR spectra were acquired on a 9.4 T Bruker MRI scanner with commercially available ^{19}F/^{1}H MRI coils.

Detection of HClO and Other Analytes with Probe 1 by ^{19}F NMR.

Probe **1** (final concentration 150 μM) treated with various analytes (final concentration ~0.45 mM) in a PBS buffer (50 mM, pH 7.4) at 25 °C for 5 min and subjected to ^{19}F NMR with the aforementioned parameters. $ONOO^-$ was generated by mixing $NaNO_2$ with H_2O_2, HO• was made by mixing $(NH_4)_2Fe(SO_4)_2$ with H_2O_2, ROO• was made by dissolving 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) in water. Other analytes were purchased from commercial sources and used as received.

Phantom Imaging with ^{19}F MRI.

Probe **1** (final concentration as indicated) was treated with HClO (1 equiv), or HClO (1 equiv) + taurine (1.2 equiv) for 13.5 min. The resulting solution was subjected to ^{9}F MRI. A FLASH sequence was used to acquire the ^{19}F MRI phantom images with the following parameters: TR/TE = 400 ms/3.3 ms, flip angle = 30°, FOV = 4 × 4 cm^3, the slice thickness = 15 mm, Matrix = 32 × 32 and 64 average (NA=64). The total experiment time was about 13.5 min.

Detection of HClO in Cells with ^{19}F NMR. SMMC-7721 cells (~2×10^6) were trypsinized and collected into a 1.5 mL centrifuge tube. After incubated with Probe **1** (Probe **1** final concentration: 1.5 mM) at 37 ℃ for 4 h and various stimuli including PBS, H_2O_2 (5 equiv. or 10 equiv.), HClO (1 equiv.) + taurine (1.2 equiv.), or HClO (1 equiv.) for 30 min, the cells were centrifuged (600 × g, 3 min) and washed three times with PBS. Then cells were lysed with 300 μL of radio immunoprecipitation assay (RIPA) lysis buffer for 30 min, and the supernatants were collected for ^{19}F NMR.

Imaging of HClO in Mice with ^{19}F MRI. Animal experiments were conducted according to the protocols approved by the Institutional Animal Care and Use Committee of Xiamen University. A nude mouse was subject to subcutaneous injections of Probe **1** (150 μL, 10 mM in PBS (50 mM, pH 7.4)) to both the right and left flank followed by an injection of HClO (25 μL 1 mM in PBS (50 mM, pH 7.4)) to the right flank. Then the mouse was subjected to ^{1}H MRI and ^{19}F MRI. A FLASH sequence was used to acquire the ^{19}F MR images with the following parameters: TR/TE = 400 ms/3.3 ms, flip angle = 30°, FOV = 4 × 4 cm^{3}, the slice thickness = 15 mm, Matrix = 32 × 32 and 64 average (NA=64). The total experiment time was about 13.5 min.

Table. S1. T_1 and T_2 relaxation times of ^{19}F in DOTA-BTFBH, Probe **1** with & without HClO.

Materials	T_1/ms	T_2/ms	T_2/T_1
DOTA-BTFBH	1260	915	0.72
Probe **1**	2.1	1.1	0.524
Probe **1** + HClO	289	197	0.68

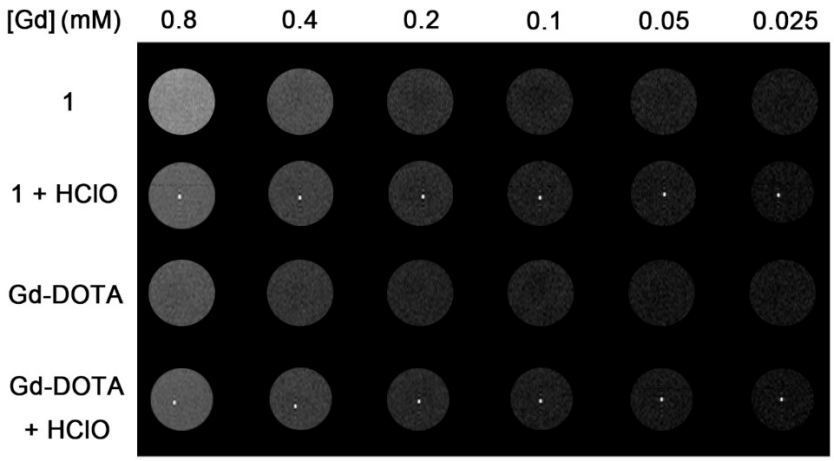


Figure S1. ^{1}H MRI phantom images of Probe **1** and Gd-DOTA with or without HClO (1 equiv) at 0.5 T.

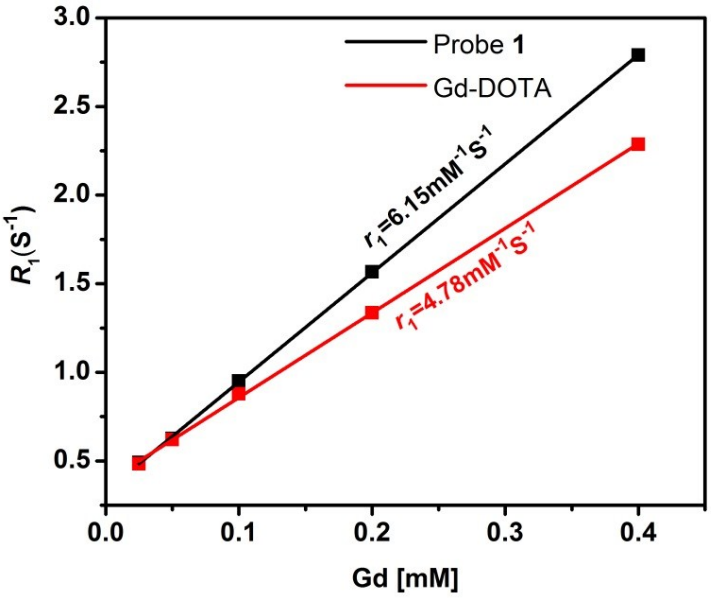


Figure S2. Relaxivities r_1 of Probe **1** and Gd-DOTA in PBS at 0.5 T.

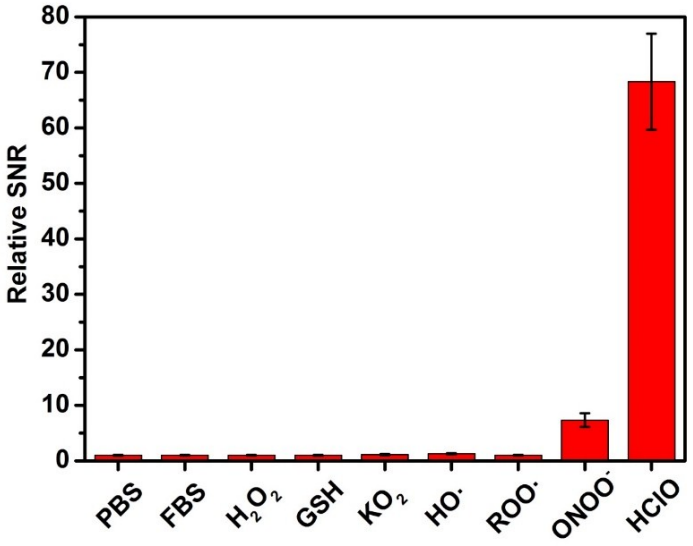


Figure S3. A plot showing the relative signal-noise ratios (SNRs) for the ^{19}F NMR spectra in Figure 2b. The SNR for the spectra of the PBS-treated sample was normalized as 1.0.

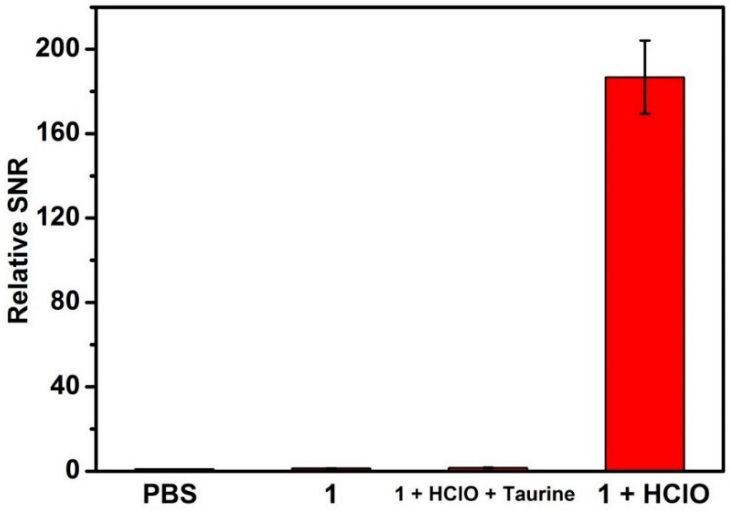


Figure S4. A plot showing the relative signal-noise ratios (SNRs) for ^{19}F MRI phantom imaging in Figure 2c.

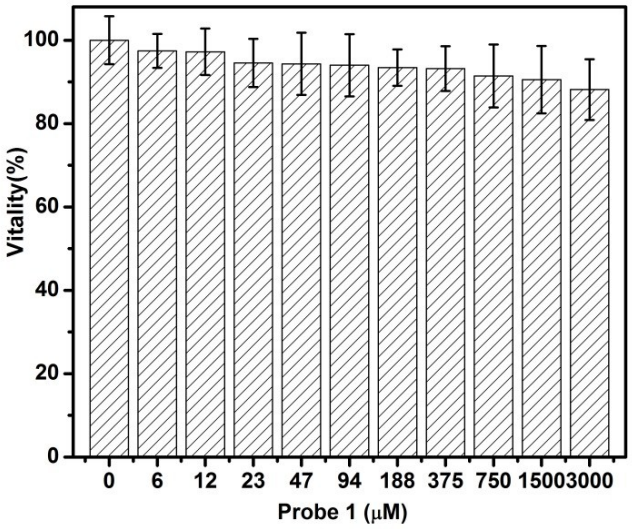
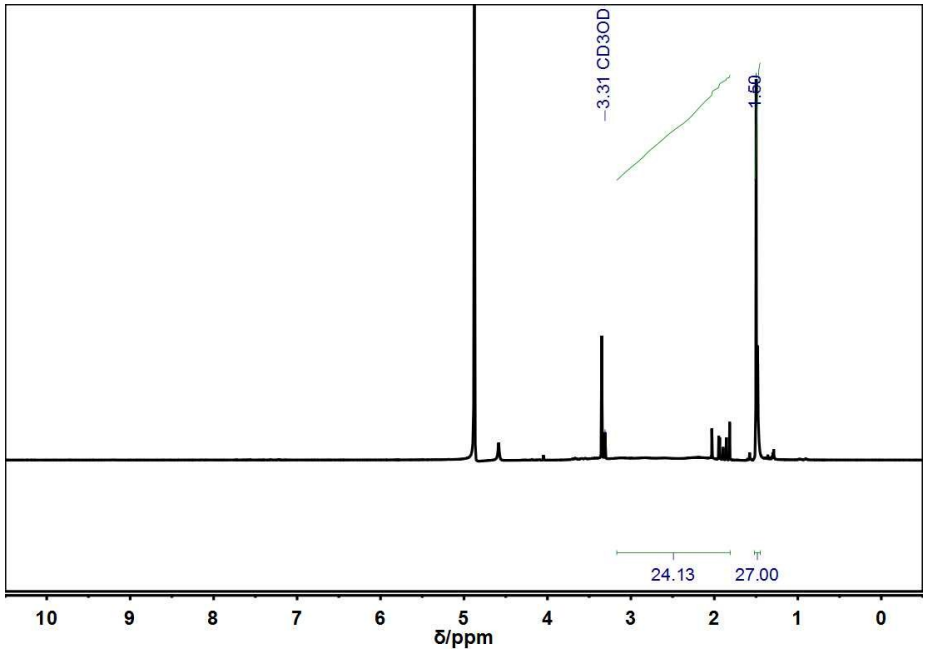
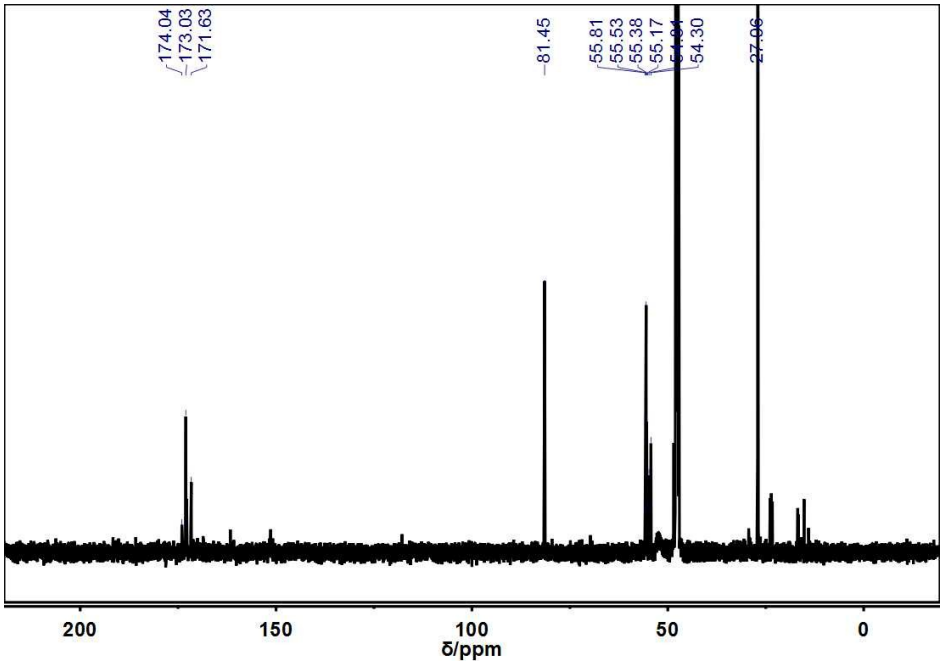


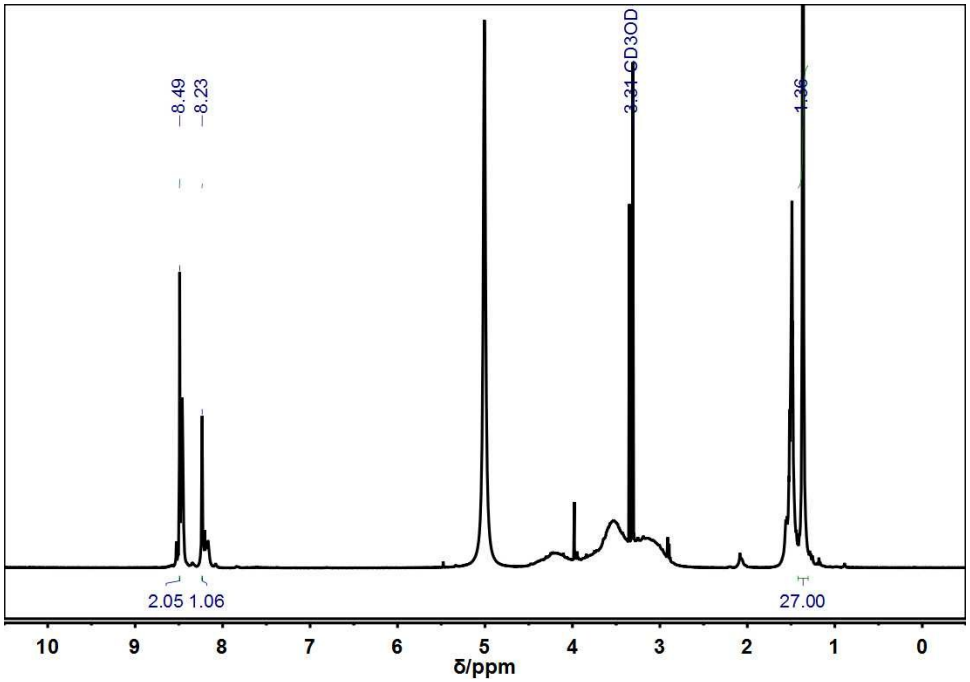
Figure S5. Cytotoxicity evaluation of Probe **1** with SMMC-7721 cells. Concentrations were with respect to Gd.

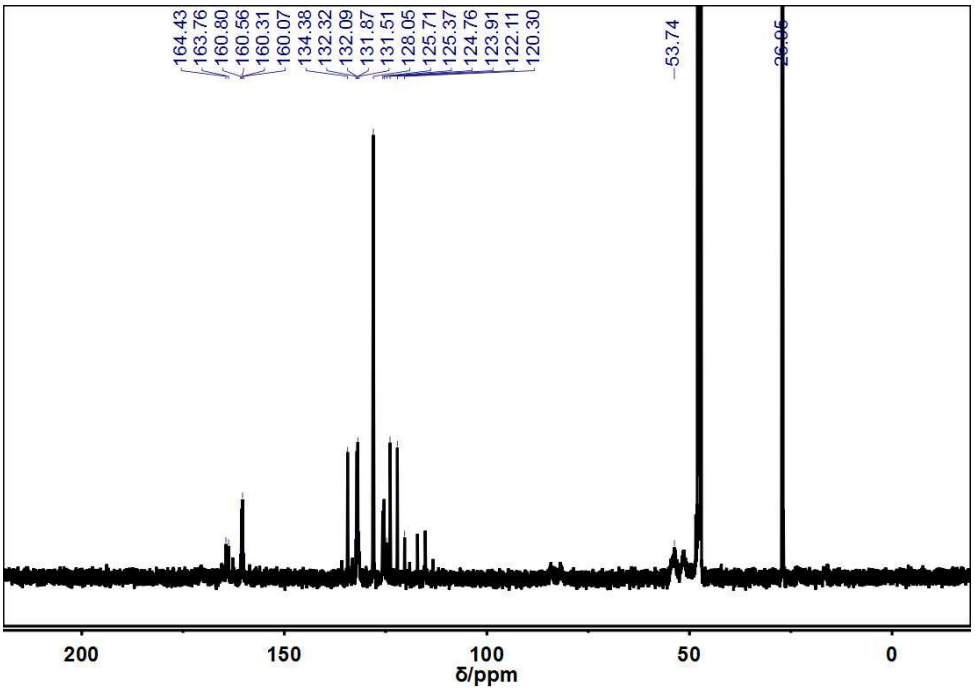
^{1}H-NMR and ^{13}C-NMR spectra of compound **4**



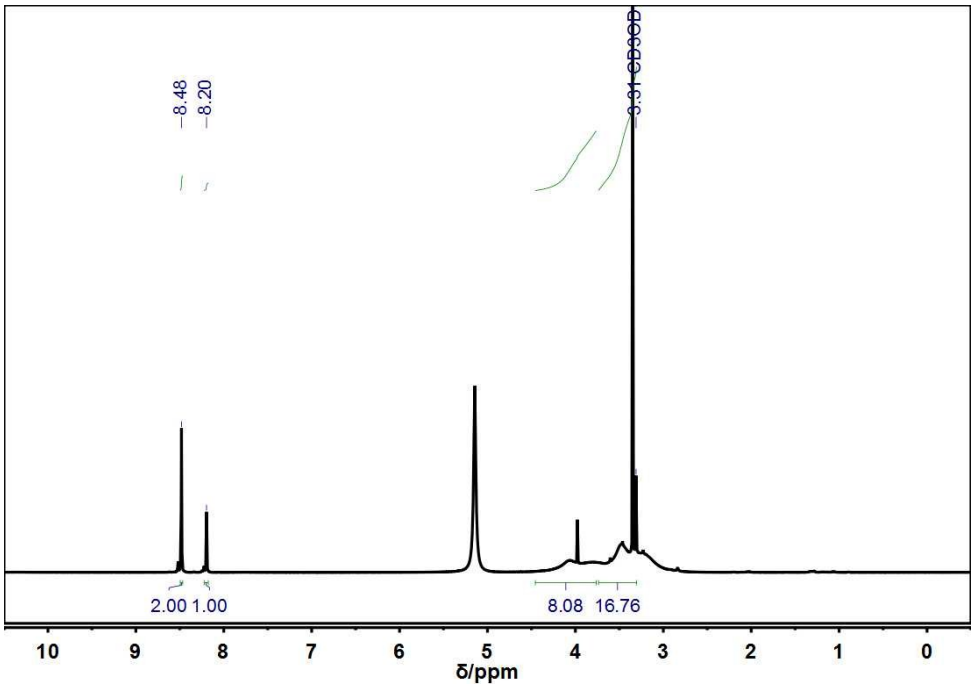


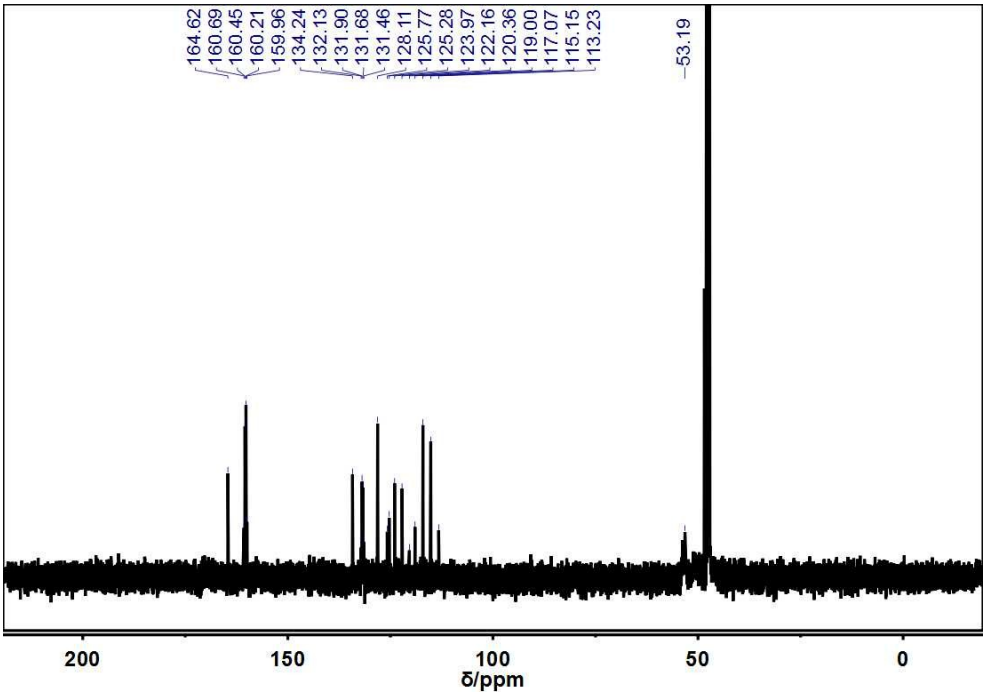
^{1}H-NMR and ^{13}C-NMR spectra of compound **5**



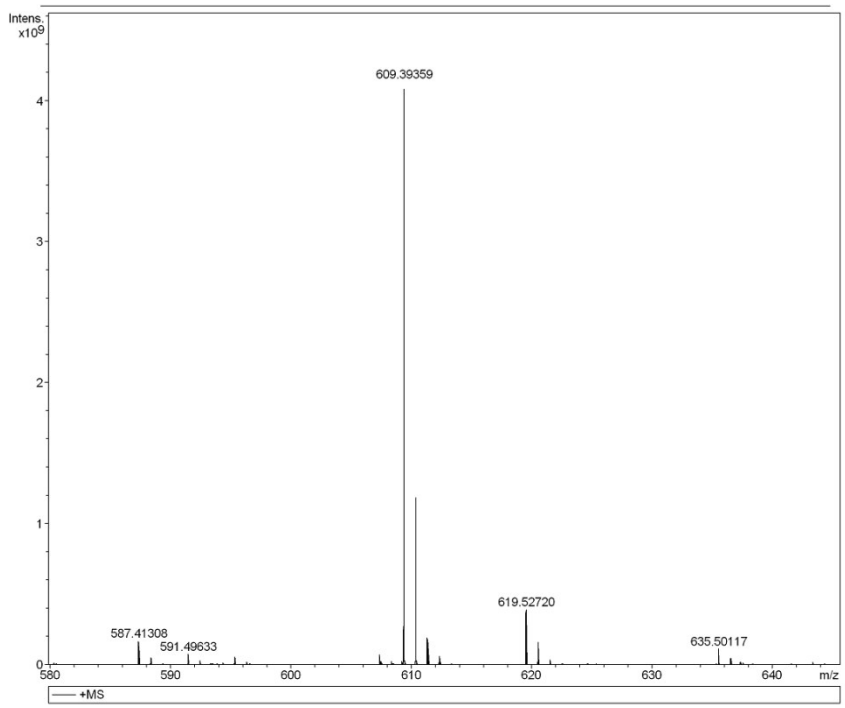


^{1}H-NMR and ^{13}C-NMR spectra of compound **6**

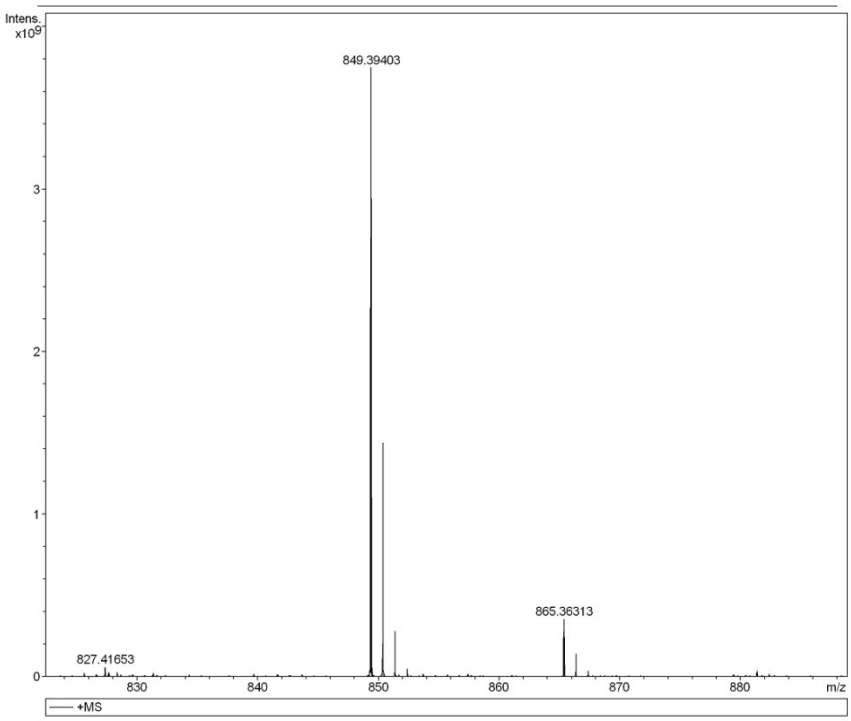




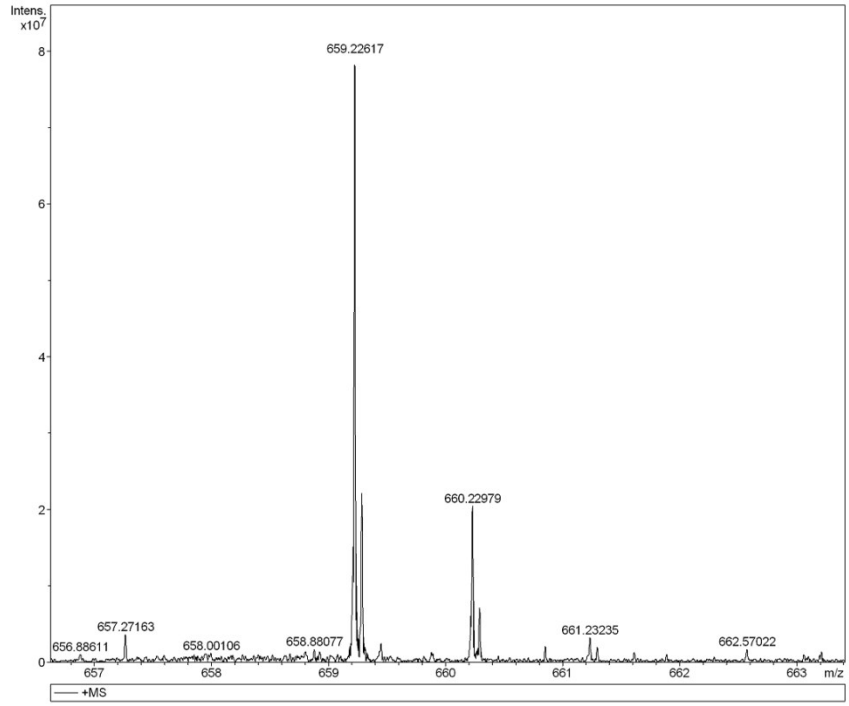
HRMS spectrum of Compound **4**



HRMS spectrum of Compound **5**



HRMS spectrum of Compound **6**



Isotopic HRMS spectrum of Probe **1**