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Supplementary Information

Geometrical Confinement Directed Albumin-Based Nanoprobes as Enhanced T_1 Contrast Agents for Tumor Imaging

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Experimental details

Synthesis of 2

tert-Butyl bromoacetate (1.3 g, 7.6 mmol, 3.3 equiv) in 10.0 mL of anhydrous chloroform was added dropwise to a mixture of 1,4,7,10-tetraazacyclododecane (cyclen) (**1**) (400 mg. 2.32 mmol) and triethylamine (2.3 g, 23.2 mmol, 10 equiv) in 40 mL of anhydrous chloroform under an argon atmosphere over 0.5 h. The reaction mixture was stirred for another 2 h, and anhydrous K_2CO_3 (160 mg, 1.16 mmol, 0.5 equiv) was added. After 24 h, the resulting solution was washed by water (3 × 40 mL), dried with anhydrous $MgSO_4$, and concentrated to give transparent oil. The crude product was purified by flash chromatography (15% v/v CH_3OH/CH_2Cl_2) on silica gel to give tris-(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (**2**) as a white powder (3.0 g, 5.8 mmol), yield: 77%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.01 (s, 1 H), 3.34 (s, 4 H), 3.26 (s, 2 H), 3.05 (s, 4 H), 2.89-2.85 (m, 12 H), 1.47 (s, 27 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.02 (2 C), 169.5, 81.7 (3 C), 58.4 (3 C), 51.0 (2 C), 49.3 (2 C), 48.5 (2 C), 47.5 (2 C), 28.1 (9 C); ESI-MS (m/z) calculated for $C_{26}H_{51}N_4O_6$ [M+H$^+$]: 515.4, found: 515.4.

Synthesis of 3

To a 100 mL round-bottom flask was added **2** (129.5 mg, 0.25 mmol), K_2CO_3 (82.3 mg, 0.6 mmol), and 20 mL of anhydrous acetonitrile. The flask was sealed and placed under a nitrogen atmosphere. To the suspension was added methyl chloroacetate (70 mg, 0.65 mmol). The resulting suspension was vigorously stirred at room temperature for 4 h. After the reaction was complete, the solid was removed by filtration, the solution was concentrated under reduced pressure. The residue was purified by flash chromatography (100% v/v CH_2Cl_2 to 20% v/v CH_3OH /CH_2Cl_2) to give **3** (131.8 mg, 0.22 mmol), yield: 90%. ^{1}H NMR (400MHz, CD_3OD): δ 3.76 (s, 3 H), 3.70-1.80 (m, 24 H), 1.52 (s, 27 H), ESI-MS (m/z) calculated for $C_{29}H_{54}N_4NaO_8$ [M + Na$^+$]: 609.4, found: 609.3.

Synthesis of 4

3 (0.43 g, 0.72 mmol) was dissolved in neat ethylenediamine (0.25 mL, 0.23 g, 3.74 mmol) and the resulting solution was stirred at room temperature for 72 h.

Ethylenediamine was removed under reduced pressure and the residue was dried under vacuum to give a light yellow foam. The crude product was purified by flash chromatography with (100% v/v CH_2Cl_2 to 50% v/v CH_2Cl_2/MeOH) to give **4** as a white foam (0.4 g, 0.65 mmol), yield: 93%. ^{1}H NMR (400 MHz, CD_3OD): δ 3.67–2.38 (m, 28 H), 2.26 (*t*, 2 H), 1.91 (s, 1 H) 1.50 (s, 27 H); ^{13}C NMR (100 MHz, CD_3OD): δ 171.47, 170.47, 170.30 (2 C), 81.42 (3 C), 56.58, 55.51 (3 C), 52.19 (2 C), 51.27 (4 C), 50.89 (2 C), 40.37, 39.54, 27.13 (9 C). ESI-MS (m/z) calculated for $C_{30}H_{58}N_6NaO_7$ [M + Na^+]: 637.4, found: 637.4.

Hematology and Histological Examination

BALB/c mice were randomly separated as control group (*n* = 4) and experimental groups (*n* = 4). The doses of Ibu-Gd-BSA NPs and Ibu-Gd for the experimental groups were 0.1 mmol Gd^{3+}/kg mouse body weight. The mice of the control group were injected with the same volume of PBS solution. After two weeks, blood was collected from the orbital sinus to evaluate the liver function. Three important blood biochemistry indicators including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured. After that, the main organs including heart, liver, spleen, lung, and kidneys were collected, sectioned and stained with hematoxylin and eosin. The histological sections were analyzed for the study of *in vivo* toxicity.

***In vivo* MR blood vessel imaging**

All studies involving animals were approved by the Animal Care and Use Committee of Xiamen University. Sprague-Dawley (SD) Rats (180~220 g) were anaesthetized by pentobarbital sodium at the dose of 40 mg/kg body weight. Dynamic T_1-weighted images were obtained before and after intravenous injection (via tail vein) of a total 0.5 mL of Ibu-Gd with a dose of 0.03 mmol Gd/kg body weight. T_1-weighted imaging was obtained on a 3 T Siemens Skyra imaging system (Angio 3D_cor_post sequence) with parameters as follows: TR = 4.61 ms, TE = 1.95 ms, field of view (FoV) = 200 mm, FoV phase = 50%, slice thickness = 0.50 mm.

Scheme S1 The synthesis of DO3A*t*Bu-NH_2 (**4**)

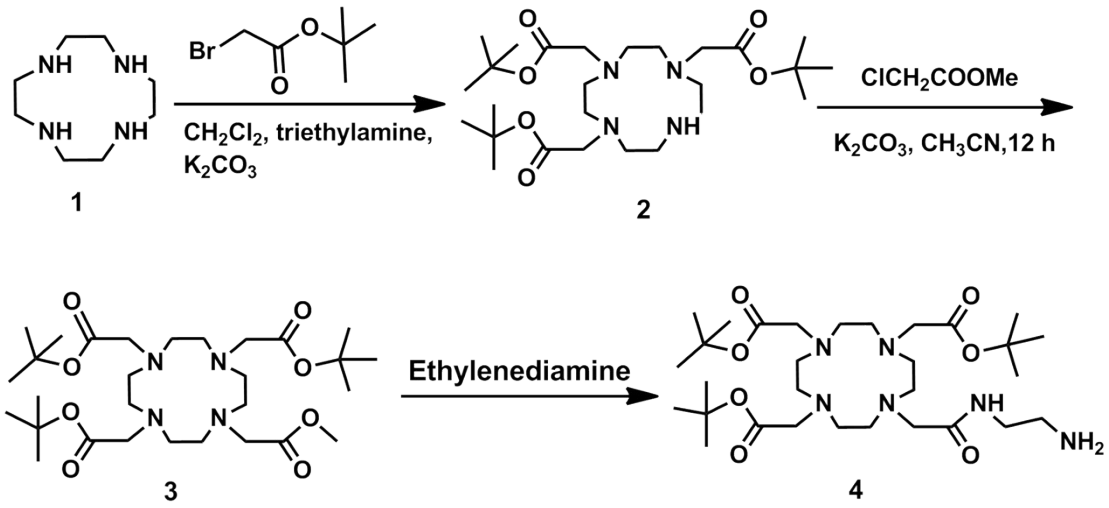
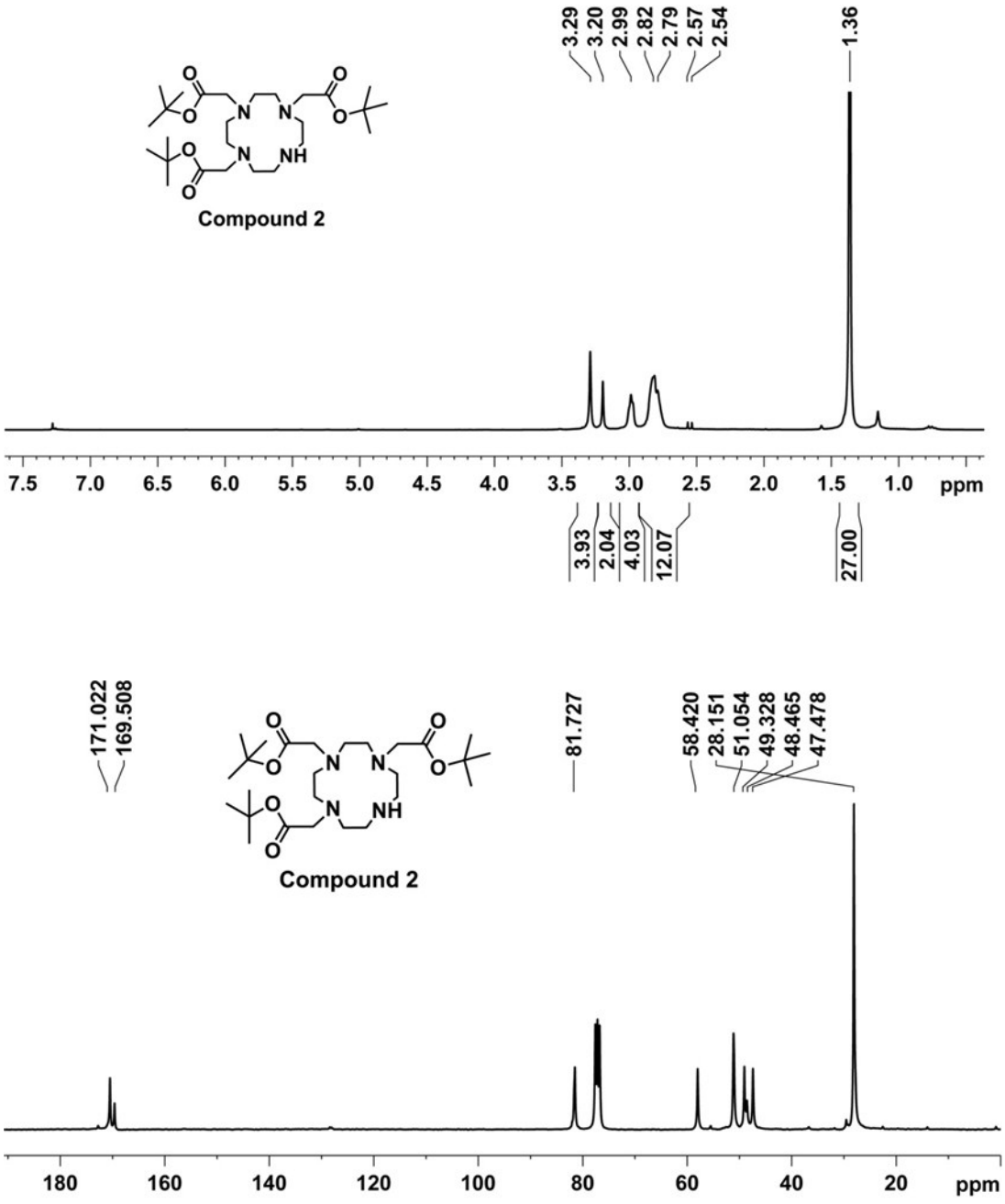
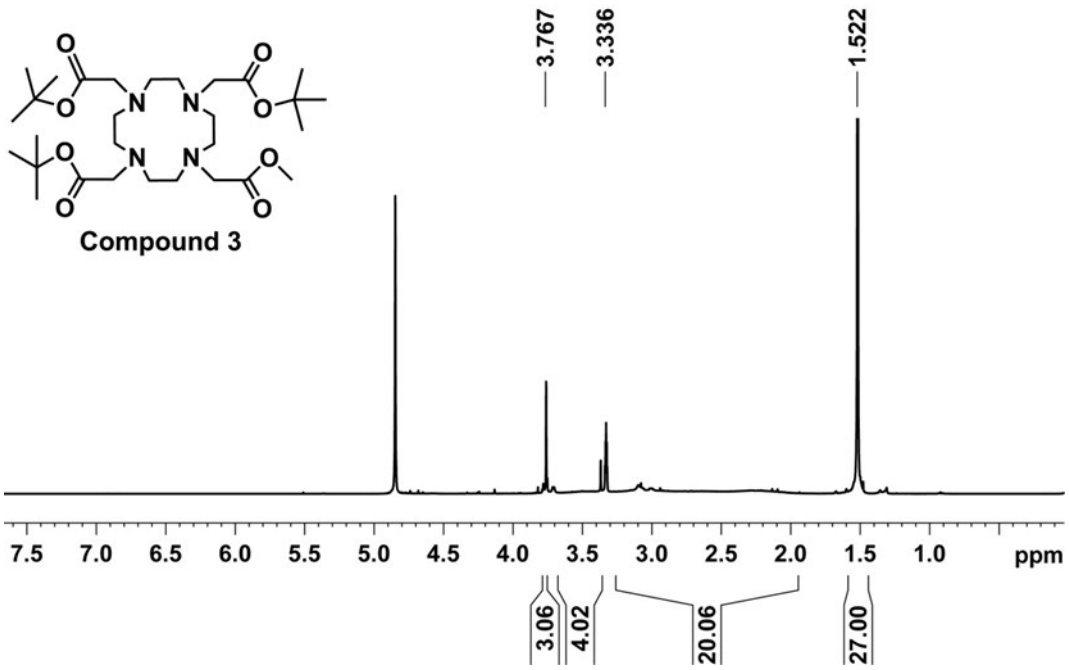


Figure S1 ^{1}H NMR and ^{13}C NMR original spectra of compound **2**~**6**.

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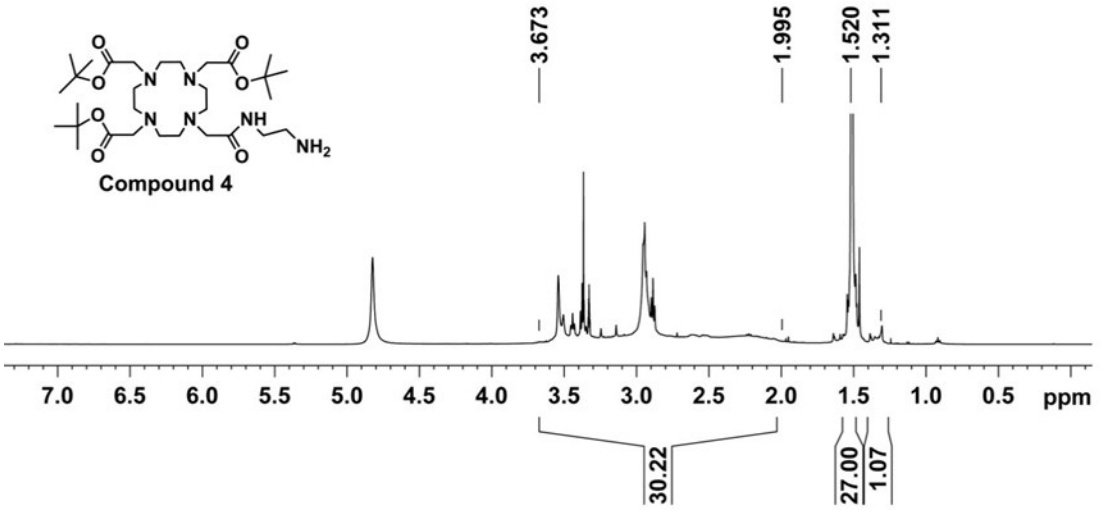
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
3.06
4.02
20.06
27.00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6.5
6.0
5.5
5.0
4.5
4.0
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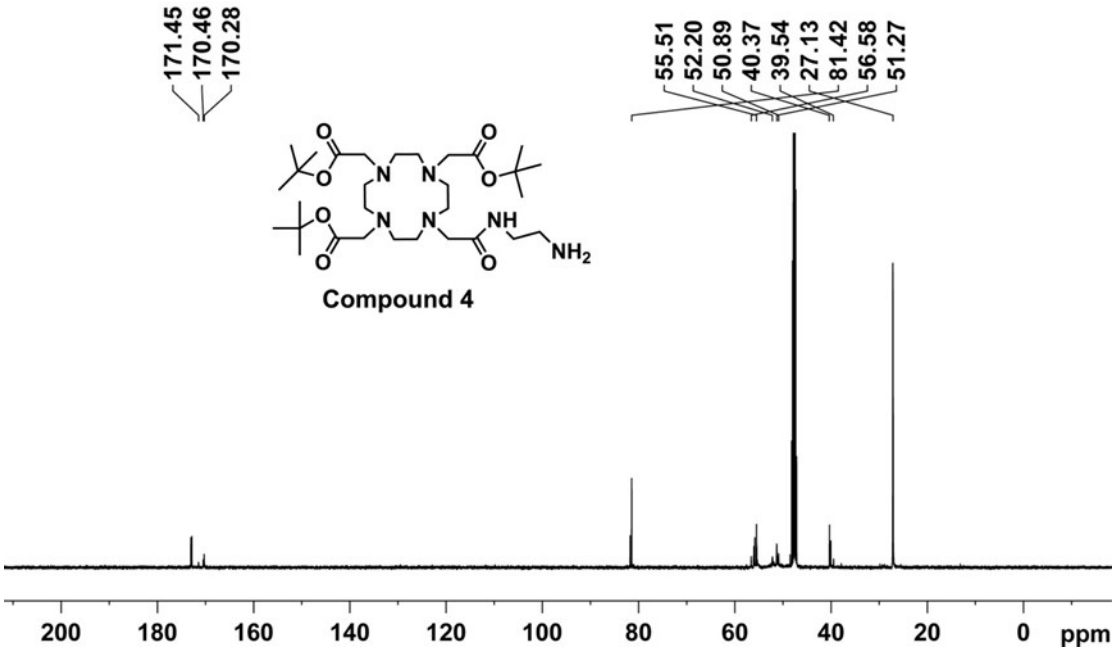
3.0
2.5
2.0
1.5
1.0
0.5
ppm
30.22
27.00
1.07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170.46
170.28
55.51
52.20
50.89
40.37
39.54
27.13
81.42
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51.27
Compound 4
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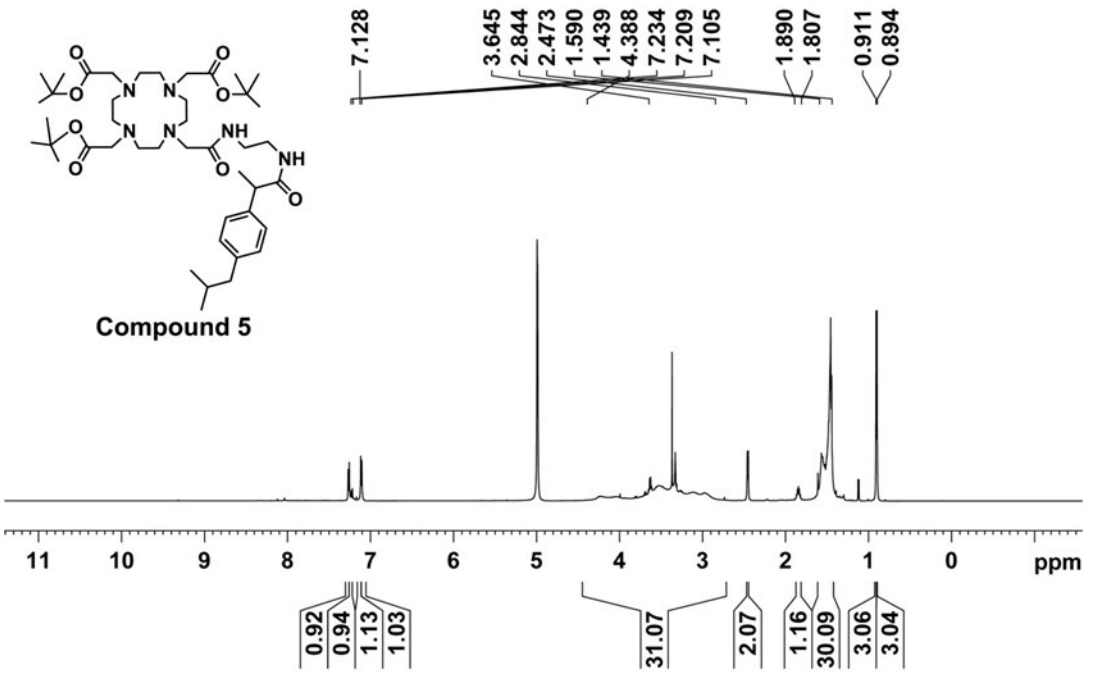
160
140
120
100
80
60
40
20
0
ppm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1.807
0.911
0.894
11
10
9
8
7
6
5
4
3
2
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1.03
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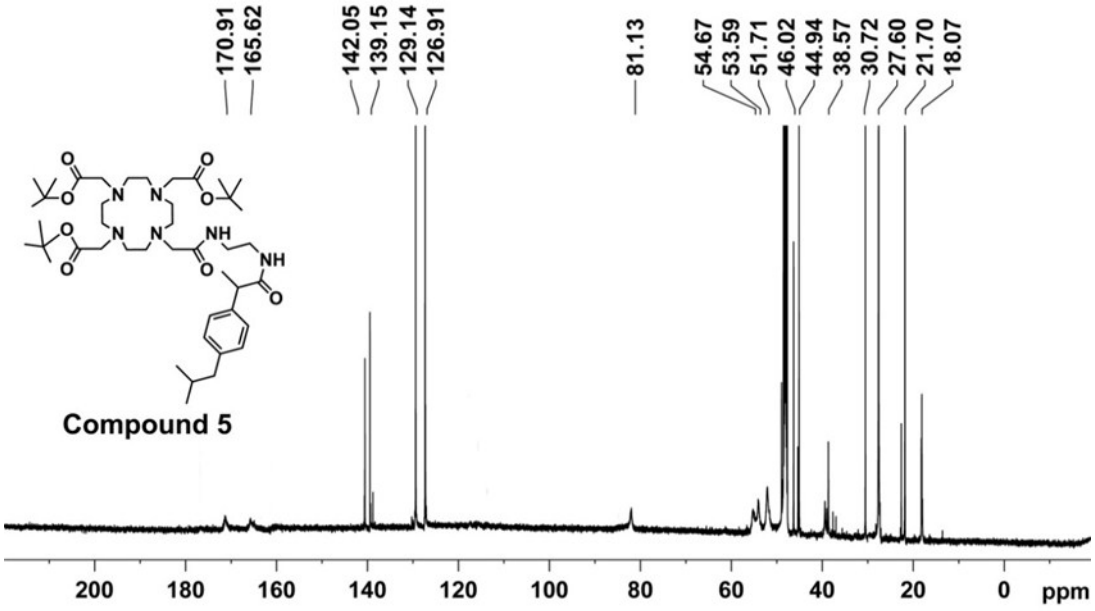
2.07
1.16
30.09
3.06
3.04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129.14
126.91
81.13
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53.59
51.71
46.02
44.94
38.57
30.72
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21.70
18.07
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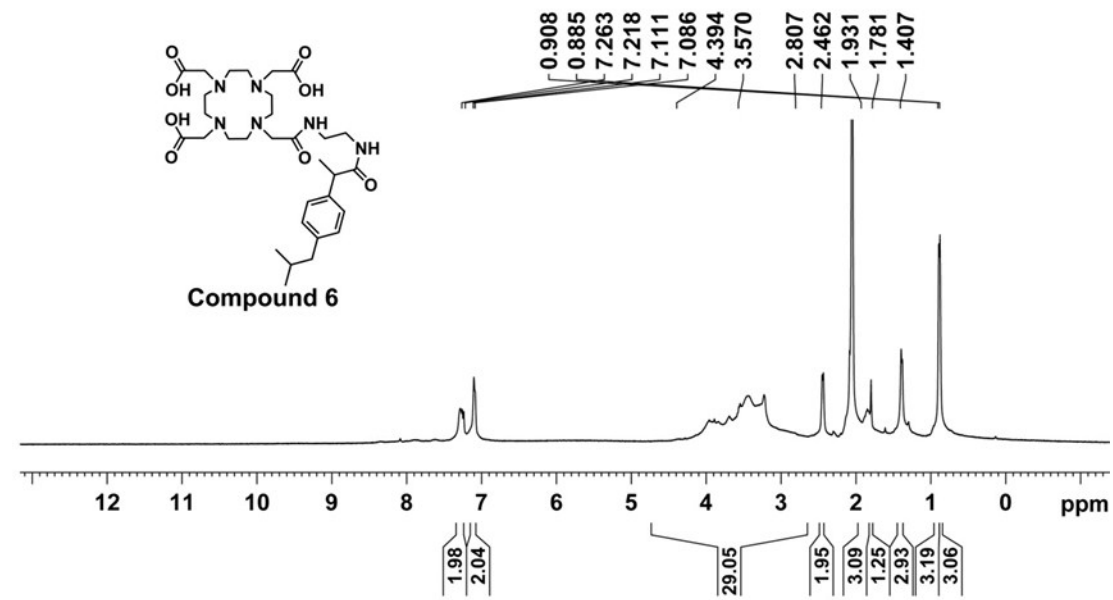
60
40
20
0
ppm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0.885
7.263
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7.086
4.394
3.570
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2.462
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1.781
1.407
Compound 6
12
11
10
9
8
7
6
5
4
3
2
1
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ppm
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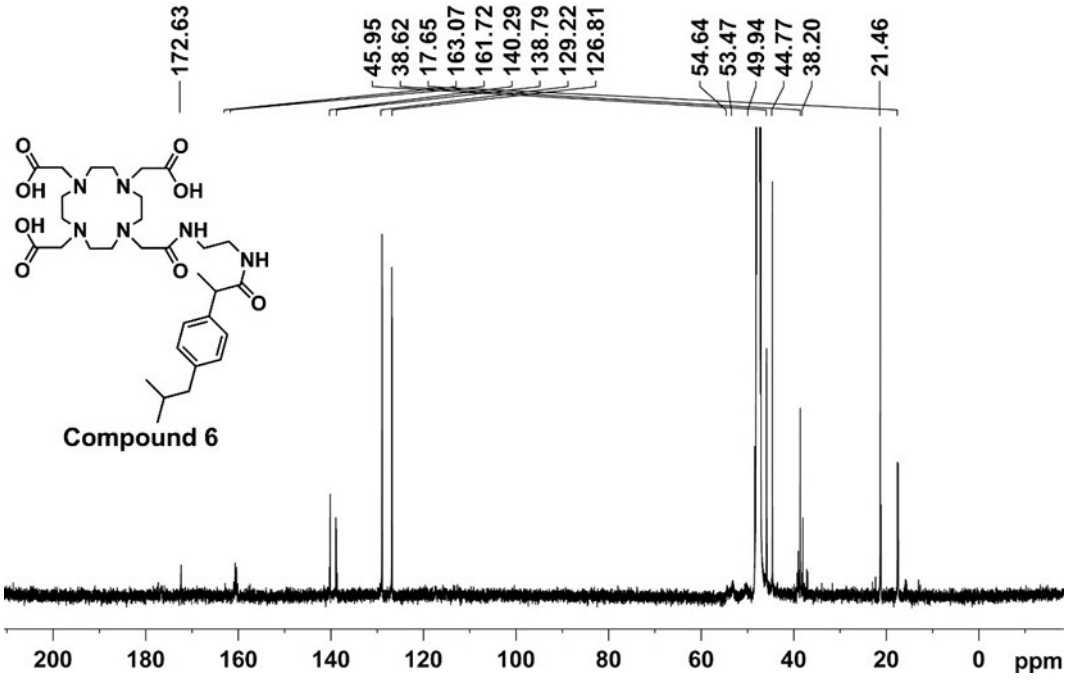
29.05
1.95
3.09
1.25
2.93
3.19
3.06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45.95
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17.65
163.07
161.72
140.29
138.79
129.22
126.81
54.64
53.47
49.94
44.77
38.20
21.46
Compound 6
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160
140
120
100
80
60
40
20
0
ppm

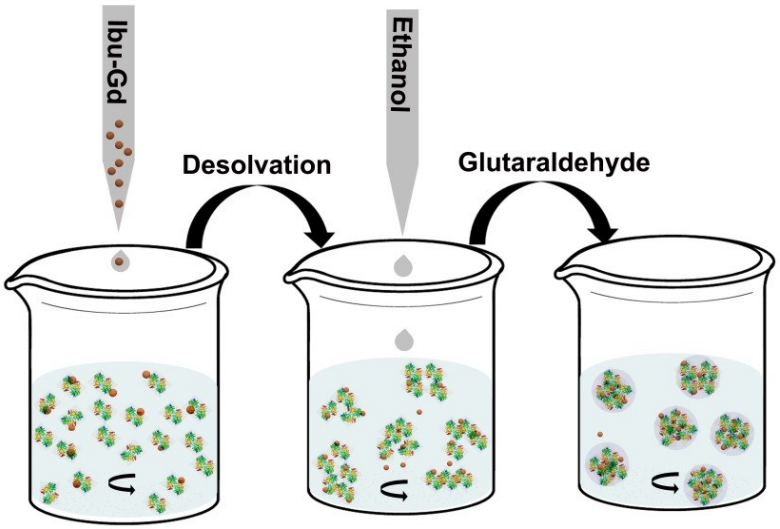

Figure S2 Schematic illustration of the synthetic procedure of Ibu-Gd-BSA NPs.

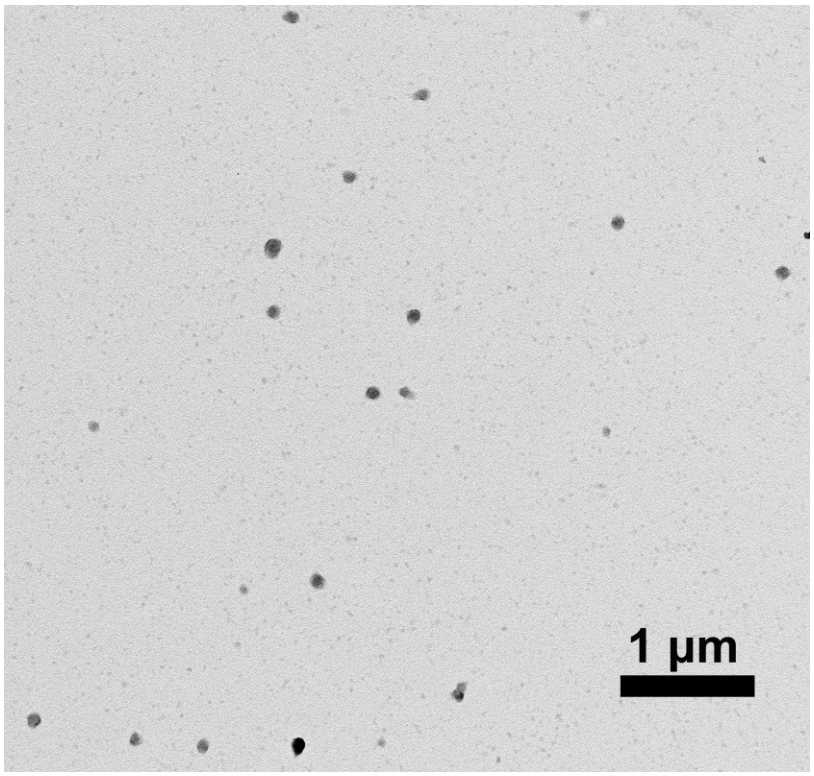

Figure S3 The representative TEM image of Ibu-Gd-BSA NPs at low magnification.

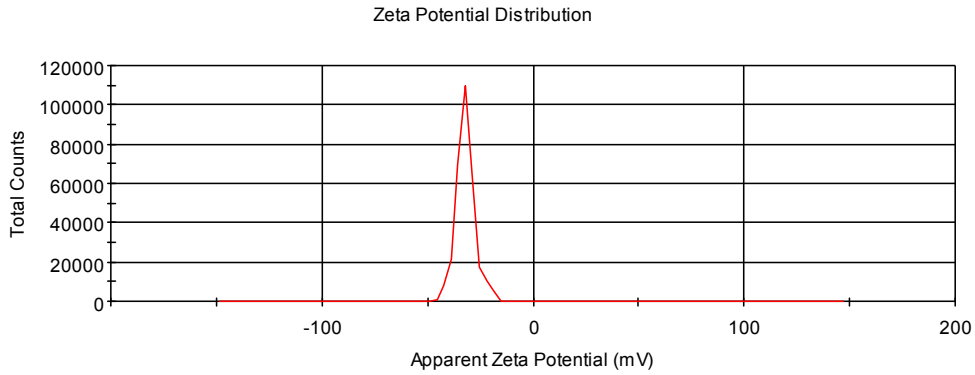

Figure S4 The ζ-potential of Ibu-Gd-BSA NPs in aqueous solution.

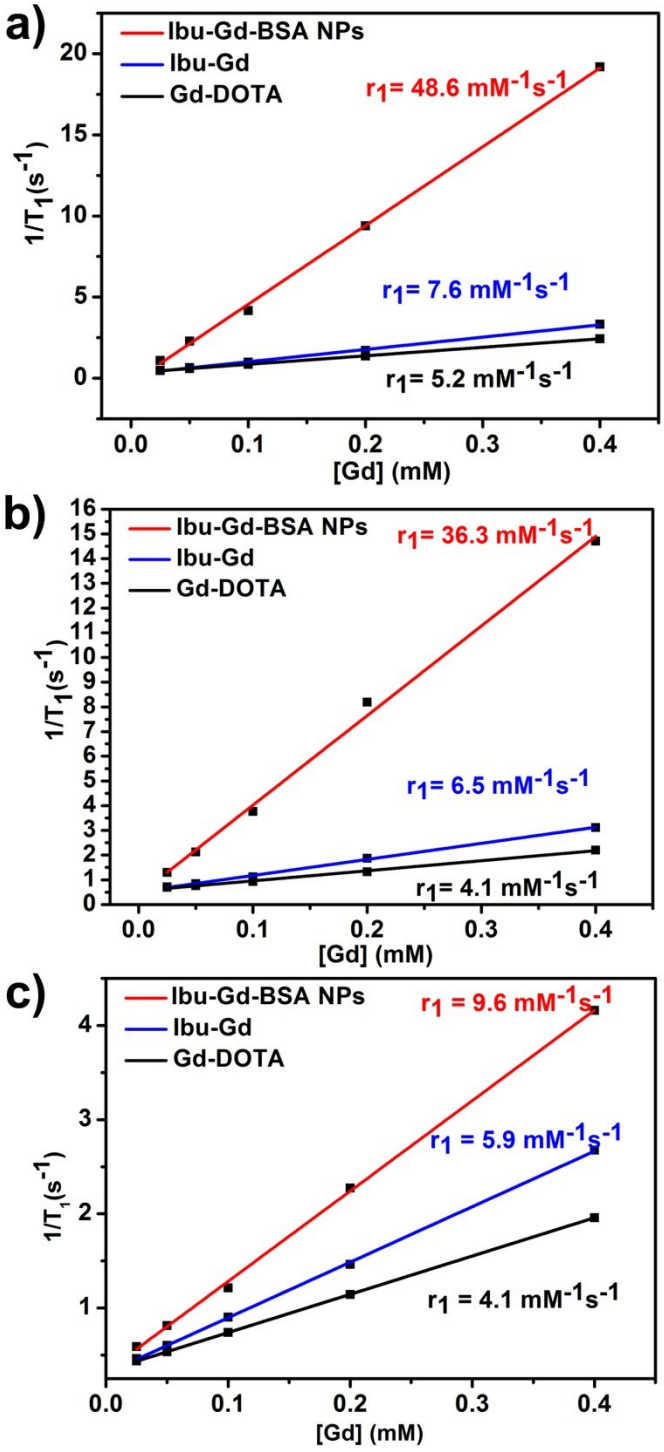

Figure S5 Relaxivities of Ibu-Gd-BSA NPs, Ibu-Gd, and clinical contrast agent Gd-DOTA at (a) 0.5 T, (b) 1.5 T, (c) 7 T.

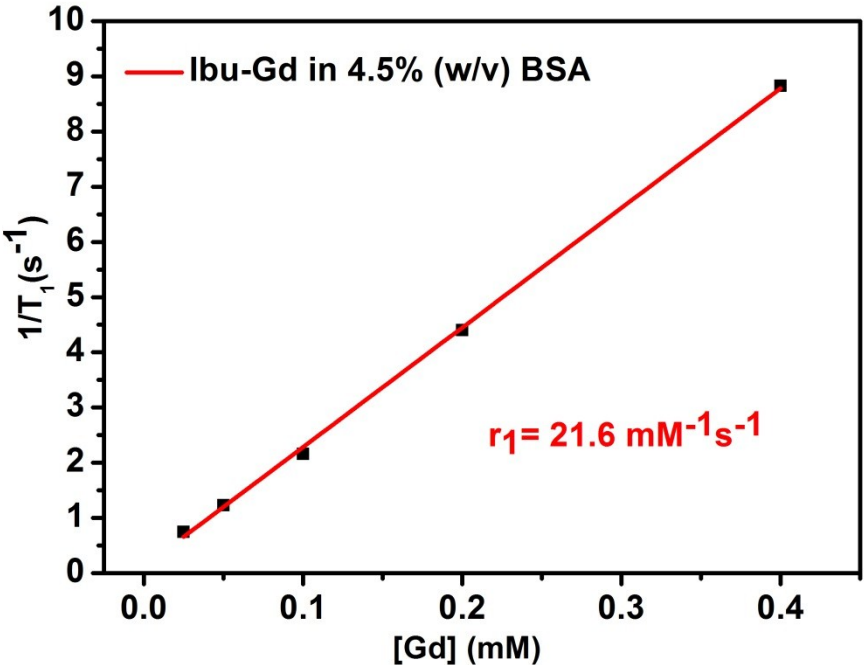

Figure S6 Relaxivity of Ibu-Gd in 4.5% (w/v) BSA aqueous solution at 0.5 T.

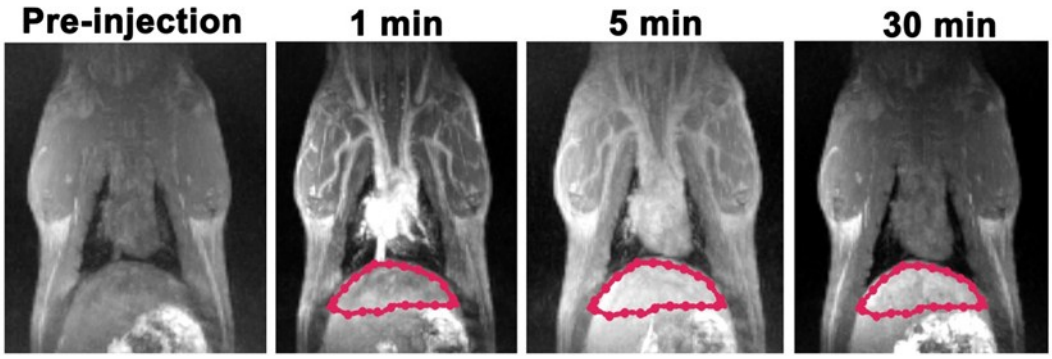

Figure S7 T_1-weighted images of SD rats pre-injection and post-injection (p.i.) of Ibu-Gd at a dose of 0.03 mmol Gd^{3+}/Kg rat body weight, the liver regions are indicated by the red circle.

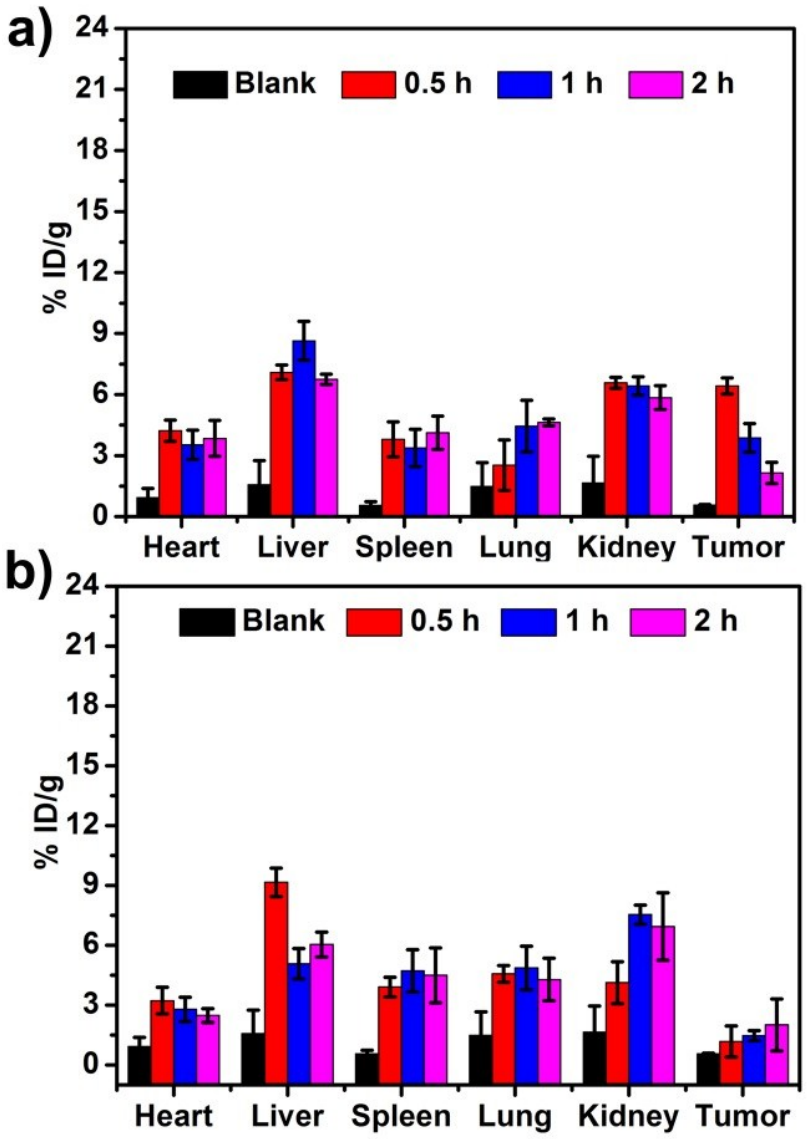

Figure S8 Biodistribution of (a) Ibu-Gd-BSA NPs and (b) Ibu-Gd in mice. ICP-MS analysis of Gd^{3+} in mouse organs (including heart, liver, spleen, lung, kidney, and tumor) at different time points after intravenous injection of Ibu-Gd-BSA NPs or Ibu-Gd (at a dose of 0.03 mmol Gd/kg mouse body weight. All data were shown as mean ± S.D., n = 4/group).

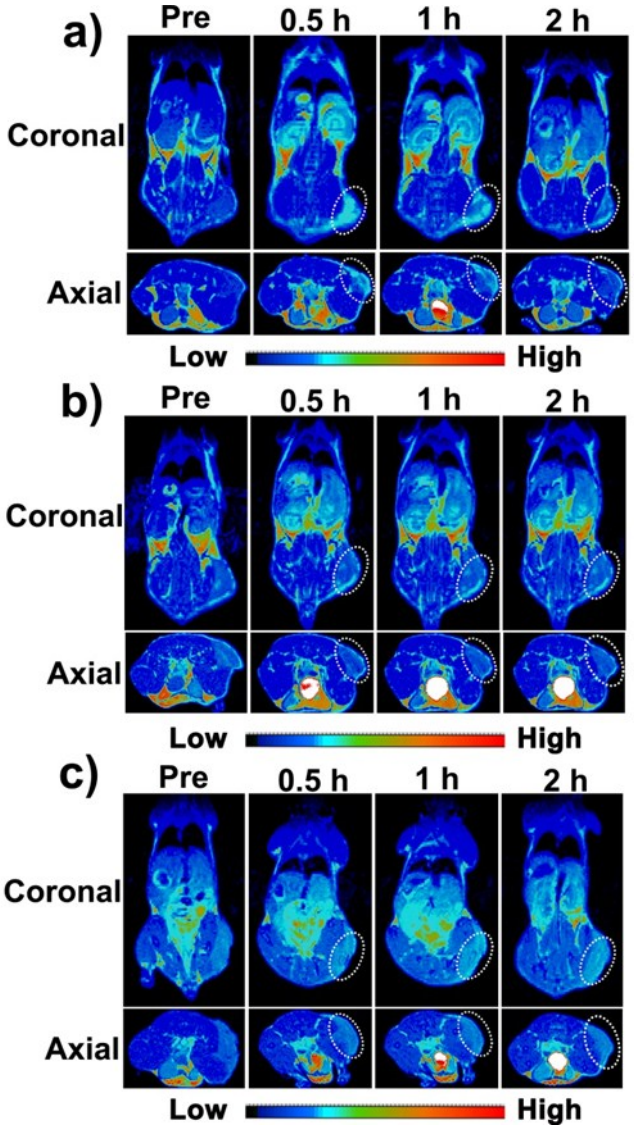

Figure S9 T_1-weighted MR imaging and quantitative analysis of subcutaneous H22 tumors after intravenous injection of Ibu-Gd-BSA NPs, Ibu-Gd, or Gd-DOTA. T_1-weighted MR false-color images on coronal and axial planes of tumor-bearing BALB/C mice before and after intravenous injection of (a) Ibu-Gd-BSA NPs, (b) Ibu-Gd, or (c) Gd-DOTA (0.03 mmol Gd/kg mouse body weight) at 0.5, 1, and 2 h.