Supporting Information

Electrostatic Self-Assembled Nanoparticles based on Spherical Polyelectrolyte Brushes for Magnetic Resonance Imaging

Weiqiao Qian[a], Qin Zhu[a], Bing Duan[b], Weijun Tang[c], Yuan Yuan[b], and Aiguo Hu*[a]

[a] *Shanghai Key Laboratory of Advanced Polymeric Materials, School of Materials Science and Engineering, East China University of Science and Technology, Shanghai, 200237, China. E-mail: hagmhsn@ecust.edu.cn; Fax: +86-21-64253037; Tel: +86-21-64253037.*

[b] *The State Key Laboratory of Bioreactor Engineering East China University of Science and Technology, Shanghai, 200237, China.*

[c] *Department of Radiology, Huashan Hospital Affiliated to Fudan University, Shanghai, 200040, China.*

Matrials and Methods

All the solvents and chemicals were purchased from commercial resources and used without further purification except for N,N-dimethylformamide (DMF), which was dried over calcium hydride and distilled under vacuum before used. Ultra-pure water was used for all characterizations, DTPA bisanhydride (DTPAA) and SPB were synthesized according to the literature procedures.[1, 2]

NMR spectroscopy

^{1}H (400 MHz), ^{1}H (500 MHz) spectra and ^{13}C NMR (100 MHz) spectra was tested by Super Conducting Fourier NMR Spectrometer in $CDCl_3$ or D_2O, with tetramethylsilane (TMS) as a reference.

Mass spectroscopy

High-resolution electrospray ionization mass spectrometry (ESI-HRMS) was achieved on a Micromass LCTTM system (Waters Ltd, America).

The content of Gd(III)

The content of Gd(III) was measured by Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES, IRIS 1000, Varian 710ES).

Hydration number measurement.

Samples with same concentration (5 mM) for europium complexes Eu-DTPA-NO-C_4 but different volumetric ratios of H_2O versus D_2O (2:8, 4:6, 5:5, 6:4, 8:2, 10:0) were prepared. Europium luminescence intensities were measured on a BioTek Synergy™ automatic microplate reader with a 50 μs interval at 616 nm for Eu

(excitation at 395 nm). The decays of luminescence intensities followed systematically monoexponential laws and were analyzed as single-exponential decays to calculate the europium luminescence lifetimes (τ) which was plotted as 1/τ versus χ. (i.e., $\chi=V_{H2O}/V$ where V, V_{H2O} and V_{D2O} are the total volume of H_2O and D_2O, the volume of H_2O and the volume of D_2O, respectively). Then by the method of extrapolation, 1/τ of H_2O versus D_2O at 0:10 ($1/\tau_{D2O}$) was founded and hydration number was calculated using the following equation:

$$q = 1.05[\tau_{H2O}{}^{-1} - \tau_{D2O}{}^{-1}]\text{-}0.25$$

T_1 relaxivity

T_1 relaxivity measurements were performed at a 1.5T GE SIGNA EXCITE at ambient temperature, the samples were diluted to different concentrations in 1.5 mL centrifuge tubes. For T_1 measurement, these samples were imaged collectively with a high-resolution inversion recovery pulse sequence (repeat time (T_R) =1600 ms, echo time (T_E) = 9 ms, inversion time (T_I) = 50, 100, 200, 300, 400, 600, 700, 900, 1200, 1500 ms, field of view = 150 mm × 150 mm, Matrix = 320 × 320). The resulting images were analyzed on a pixel-by-pixel basis to a single exponential. The T_1 values were calculated upon the average over at least 45 pixel in the center of every sample, then plot $1/T_1$ versus the concentration of Gd(III) [Gd^{3+}] using Origin, The relaxivity values for assemblies SPB-C_4 were calculated using the following equation:

$$\frac{1}{T_1} = \frac{1}{T_{1d}} + r_1[Gd^{3+}]$$

The slope of the curve represents the relaxivity, r_1.

DLS and Zeta potential

DLS measurements and Zeta potential were both carried out with a zetasizer analyzer (Nano ZS, Malvern). DLS measurements were measured at a fixed scattering angle of 90°. The particle size distribution was calculated as number-weighted Gaussian distribution by ZPW388 software.

TEM

TEM images were obtained on a JEOL JEM 2100 microscope operating 200 kV. TEM samples were prepared by dropping the original samples on the carbon/copper grid, and allowed the solvent to evaporate slowly under ambient conditions to prepare the dry samples.

SEM

SEM images and EDS were achieved with a Field Emision Scanning Electron Microscope. The sample preparation method was the same with TEM.

Transmetalation Kinetics

The kinetic inertness of Gd-DTPA-NO-C_4 against transmetallation was evaluated in vitro in comparison with Omniscan. Firstly, 5 ml of 1 mM Gd complex in

phosphate buffer and 20μl of a 250 mM aqueous solution of $ZnCl_2$ were mixed, the mixture was stirred and samples (0.3 ml) were collected before and at 2, 4, 6, 8, 10, 24 and 48h of incubation, and then filtered. The concentration of Gd(III) in the supernatant was measured by ICP-AES. The kinetic inertness was determined as the percentage of the bound Gd(III) post-incubation to that of before incubation.

Cytotoxicity

The cytotoxicity of the SPB-0.8 were tested with human cervical carcinoma cell (HeLa Cells) as the model cell lines by the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT assay. Generally, HeLa cells were cultured in 25 mL flasks with Dulbecco's modifed Eagle's medium (DMEM) containing 10% fetal bovine serum, antibiotics (100 U mL−1 penicillin-G, and 100 μg mL−1 streptomycin) at 37 °C under a humidified atmosphere of 5% CO2/95% air. In order to test the toxicity, the cells were plated into 96-wellplates at a density of 4.0×10^3 cells per well in 0.1 mL culture medium and allowed to attach for 24 h. Then the growth media was removed and the cells were washed with PBS twice. Then DMEM medium was added into each well with SPB-0.8 in different concentrations of 20, 40, 60, 80, 100 and 150μM, after incubating cells for 48h, the MTT assay was carried out following the standard protocol. Then quantify the purple formazan in the supernatant by measuring at 492 nm absorbance in a spectrophotometer (SPECTRAmax384, Molecular Devices, USA).

Synthesis of N-(tert-butyloxycarbonyl)-2-bromoethylamine 2

2-bromoethylamine **1** (55.5mmol) and dichloromethane (DCM, 250 mL) were added into a 500 mL flask, followed by the addition of di-tert-butyl pyrocarbonate (50 mmol). The turbid reaction system was transferred to an ice-water bath, and distilled anhydrous triethylamine was added dropwise into the flask with a constant pressure funnel, the reaction mixture slowly becomes clear and transparent. It is further stirred at room temperature for 18 h. After evaporation off the solvent under vacuum, the crude product was dissolved in DCM, subsequently washed with aqueous $KHSO_3$ (3 × 20 mL) and sodium chloride (3 × 20 mL), the organic phase is collected and evaporate under vacuum to obtain the colorless solid (81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.95 (br s, 1H, NH), 3.53 (t, 2H, NCH_2), 3.45 (t, 2H, CH_2Br), 1.42 (s, 9H, $(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.7, 80.0, 42.5, 33.0, 28.5. ESI-HRMS: m/z calc. for $C_7H_{15}BrNO_2^+$: 224.0281, found: 224.0268.

Synthesis of (2-tert-butoxycarbonylamino-ethyl)-butyl-dimethyl-ammonium 3

N-(tert-butyloxycarbonyl)-2-bromoethylamine **2** (14 mmol) was added into a solution of N,N-Dimethylaminobutane (14 mmol) in acetonitrile (10 mL), the reaction mixture was stirred at 80℃ for 72 h. After evaporation off the solvent under vacuum, the crude product was recrystallized from ethanol/diethyl ether (3 mL/15 mL), the precipitation was filtered off, followed by washing with bulk ethyl acetate to remove the excessive tertiary amine. The final product was dried *in vacuo* to obtain a write

solid (85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.29 (br, 1H), 3.74 (t, 4H), 3.55 (t, 2H), 3.37 (s, 6H), 1.73 (m, 2H), 1.45 (m, 2H), 1.42(s, 9H), 1.01 (t, 3H). ^{13}C NMR (101 MHz, $CDCl_3$): δ 156.5, 80.1, 65.1, 62.8, 52.0, 35.5, 28.5, 24.8, 19.7, 13.8. ESI-HRMS: m/z calc. for $C_{13}H_{29}N_2O_2^+$: 245.2224, found: 245.2228.

Synthesis of (2-amino-ethyl)-butyl-dimethyl-ammonium 4

(2-tert-Butoxycarbonylamino-ethyl)-butyl-dimethyl-ammonium **3** (2 mmol) was added into the 20 mL Schlenk flask with trifluoroacetic acid (10 mmol), followed by adding of DCM (2 mL). The reaction mixture was stirred under nitrogen atmosphere at room temperature. After 18 h, the colorless oil (99%) was obtain by the evaporation of the solvent under vacuum. ^{1}H NMR (400 MHz, D_2O): δ 3.24 (m, 4H), 3.01 (s, 6H), 2.97 (t, 2H), 1.67 (m, 2H), 1.30 (m, 2H), 0.88 (t, 3H). ^{13}C NMR (101 MHz, D_2O): δ 163.2, 162.9, 162.6, 162.3, 119.7, 117.4, 115.1, 112.7, 64.4, 50.8, 34.0, 23.7, 18.9, 12.7. ESI-HRMS: m/z calc. for $C_8H_{21}N_2^+$: 145.1699, found: 145.1704.

Synthesis of DTPA-C_4

DTPA bisanhydride (DTPAA) (1 mmol) was dissolved in N,N-dimethylformamide (DMF) in 50 mL Schlenk flask. Subsequently, (2-amino-ethyl)-butyl- dimethyl-ammonium **4** (2 mmol) was added into the flask. The reaction mixture was stirred under nitrogen atmosphere at 60℃. After reacted for 24h, DMF was remove by vacuum distillation, the crude product was recrystallized from methanol/DCM, followed by the second recrystallization from ethanol/diethyl ether, the precipitation was collected and dry under vacuum to obtain a write solid (89%). ^{1}H NMR (500 MHz, D_2O+NaOD): δ 3.62 (t, 4H), 3.38 (t, 4H), 3.26 (t, 4H), 3.20 (s, 4H), 3.09 (s, 4H), 3.04 (m, 14H), 2.51-2.60 (m, 8H), 1.67 (m, 4H), 1.31 (m, 4H), 0.87 (t, 6H). ^{13}C NMR (101 MHz, D_2O): δ 178.8, 178.3, 174.9, 162.7, 162.4, 119.5, 117.2, 114.8, 112.5, 64.2, 61.1, 58.9, 58.1, 57.6, 52.2, 51.4, 50.6, 32.6, 23.5, 18.7, 12.5. ESI-MS: m/z calc. for $C_{30}H_{60}N_7O_8^+$: 646.4498, found: 646.4509.

Synthesis of DTPA-NO-C_4

DTPA-C_4 (0.25 mmol) was dissolved in methanol (2 mL) at room temperature, followed by addition of excessive hydrogen peroxide (3 mL). After stirring for 72 h, Pd/C catalyst was used to remove the unreacted hydrogen peroxide. Then the mixture was filtered to remove Pd/C catalyst and the filtrate was evaporated to provide a transparent oil. Followed by the recrystallization from ethanol/diethyl ether, the mixture was placed in the refrigerator overnight, then filtered and dried *in vacuo* to obtain a yellow solid (79%). ^{1}H NMR (500 MHz, D_2O+NaOD): δ 3.25-3.96 (m, 24H), 3.01-3.05 (m, 14H), 2.6-2.8 (m, 4H), 1.68 (m, 4H), 1.29 (m, 4H), 0.87 (t, 6H). ^{13}C NMR (101 MHz, D_2O): δ 179.0, 175.1, 173.1, 162.9, 162.6, 119.7, 117.4, 115.0, 112.2, 67.7, 64.5, 61.3, 50.8, 48.3, 33.9, 33.3, 33.0, 32.8, 23.7, 18.9, 12.7. ESI-MS: m/z calc. for $C_{30}H_{60}N_7O_{11}^+$: 694.4345, found: 694.4352.

Synthesis of Gd-DTPA-NO-C_4

A solution of DTPA-NO-C_4 (0.2 mmol) in methanol (6 mL) was added to the 100

mL flask combined with a solution of $GdCl_3 \cdot 6H_2O$ (0.22 mmol). Followed by the addition of excessive pyridine (2 mmol), the reaction was stirred at 55℃ for 24 h. After cooling down to room temperature, the free Gd(III) was removed by adjusting the pH of the solution to 10, and filtered the precipitation through a 0.22 mm Millipore filter, then evaporated the solvent to obtain the yellow solid. ESI-MS: m/z calc. for $[M\text{-butyl}+Na]^+$: 815.2, found: 815.2.

Preparation of SPB assemblies

Firstly, SPB was treated with 10 mM NaOH to convert carboxyl groups into carboxylate ion to give the neutral SPB. The brush layer of SPB is full of negative charge (COO^-), which is necessary for the electrostatic self-assembly.

Cationic Gd complexes were adding to the stiring solution of SPB according different charge molar ratio (Gd complexes/SPB) to afford four assemblies (SPB-0.2, SPB-0.4, SPB-0.6 and SPB-0.8). After stiring for overnight at room temperature, the mixture was dialyzed (MWCO 1000) against ultra-pure water for 48 h.

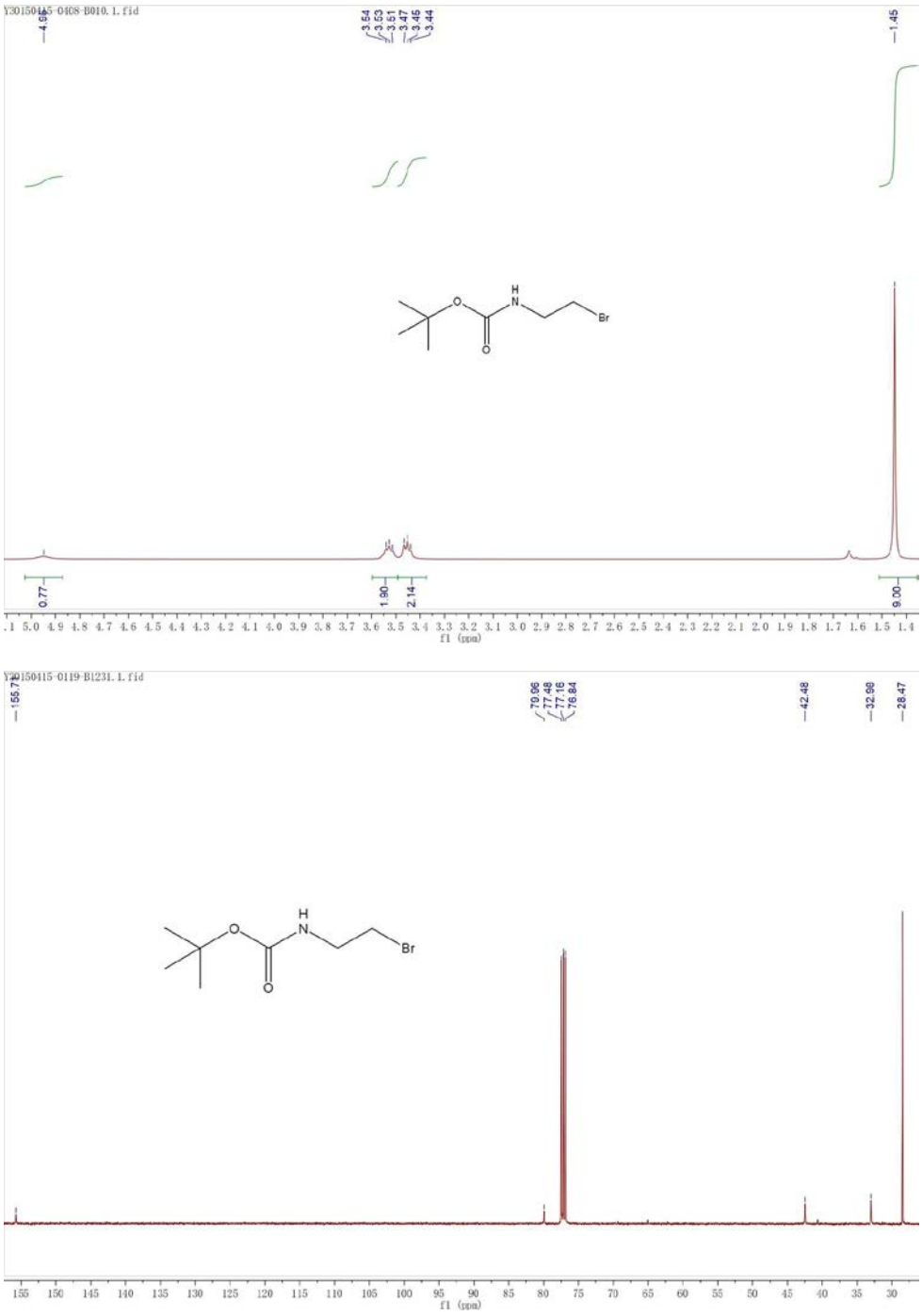

Fig. S1 ^{1}H NMR and ^{13}C NMR of spectra of compound **2**

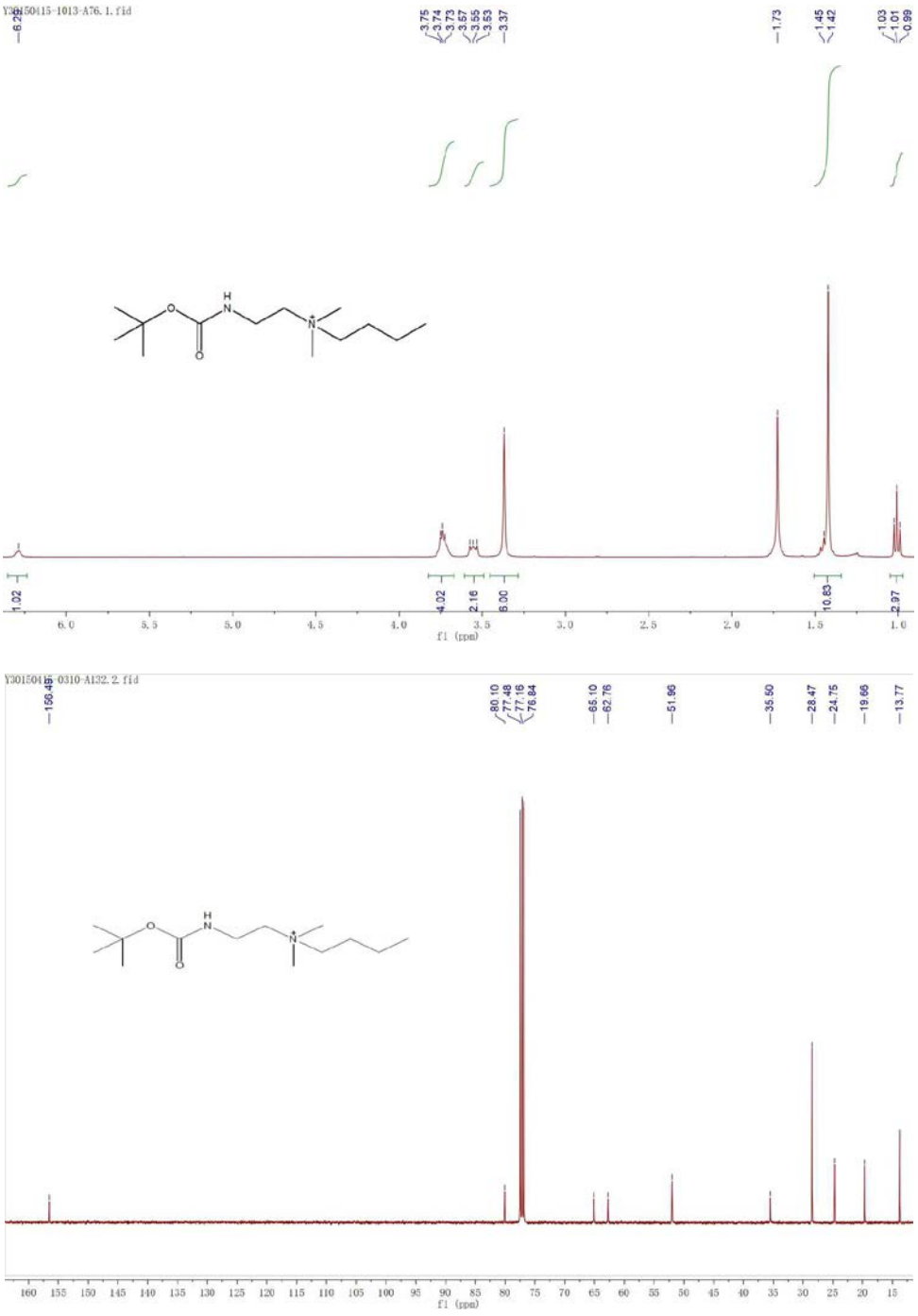

Fig. S2 ^{1}H NMR and ^{13}C NMR of spectra of compound **3**

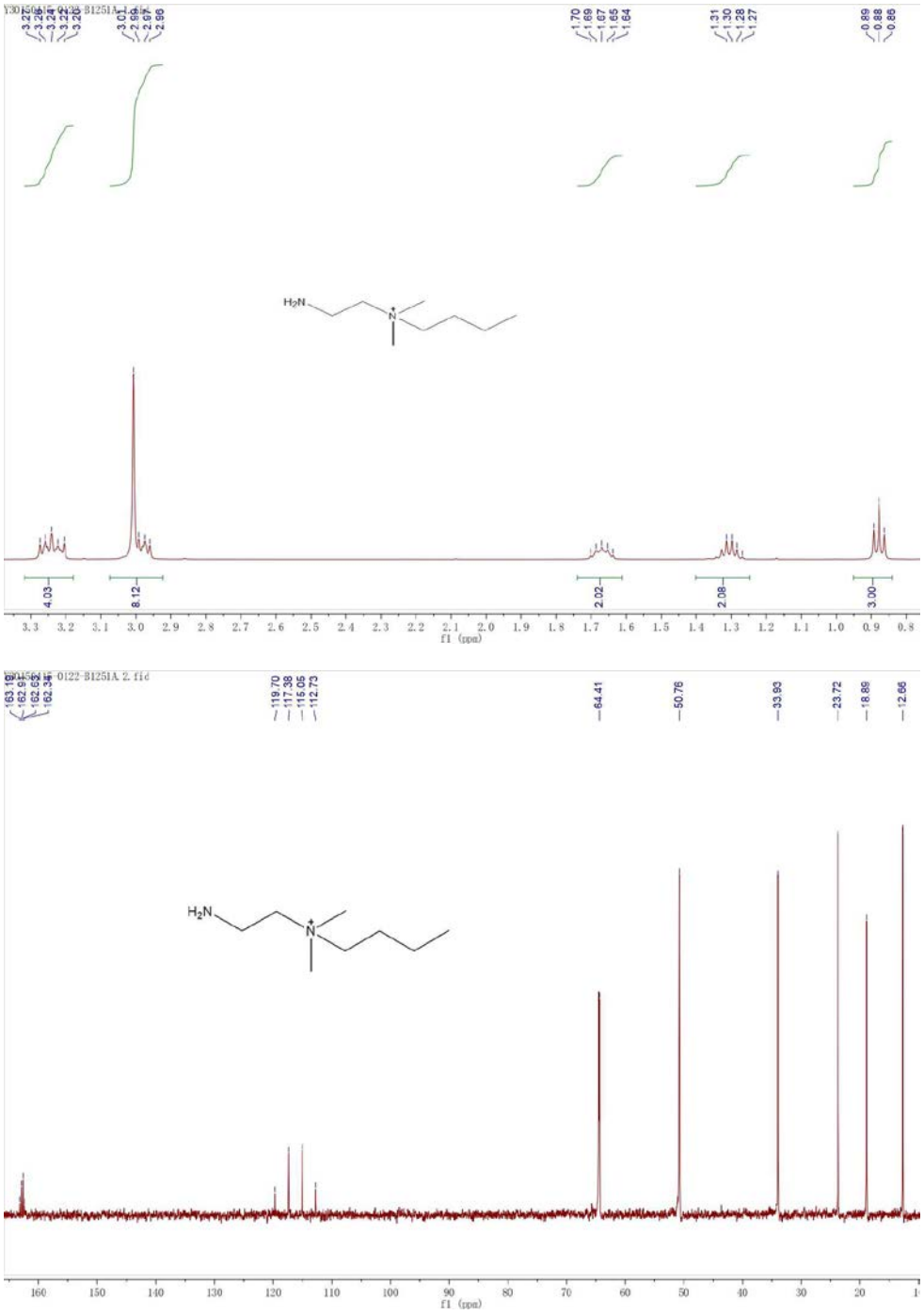

Fig. S3 ^{1}H NMR and ^{13}C NMR of spectra of compound **4**

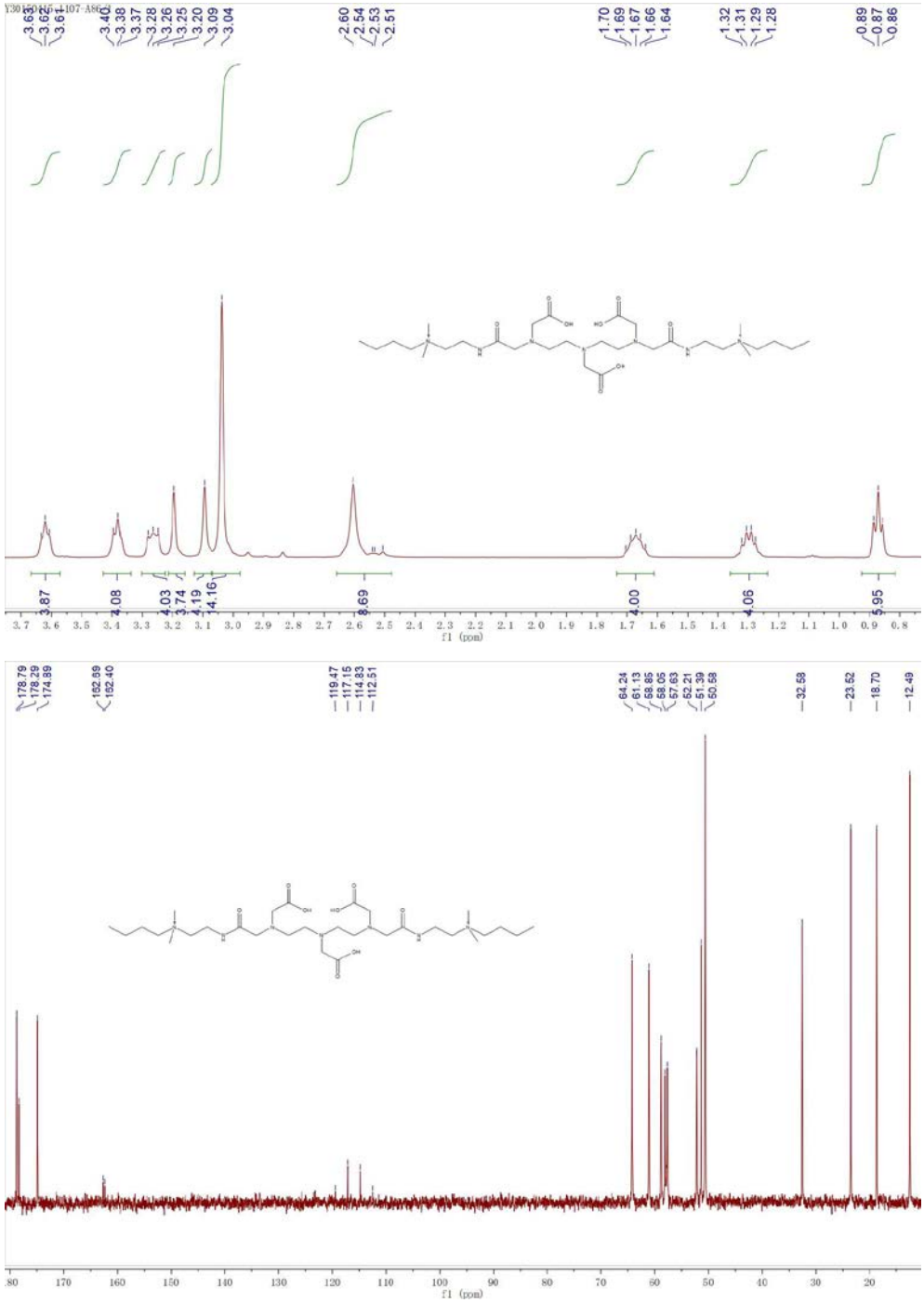

Fig. S4 ^{1}H NMR and ^{13}C NMR of spectra of compound **5**

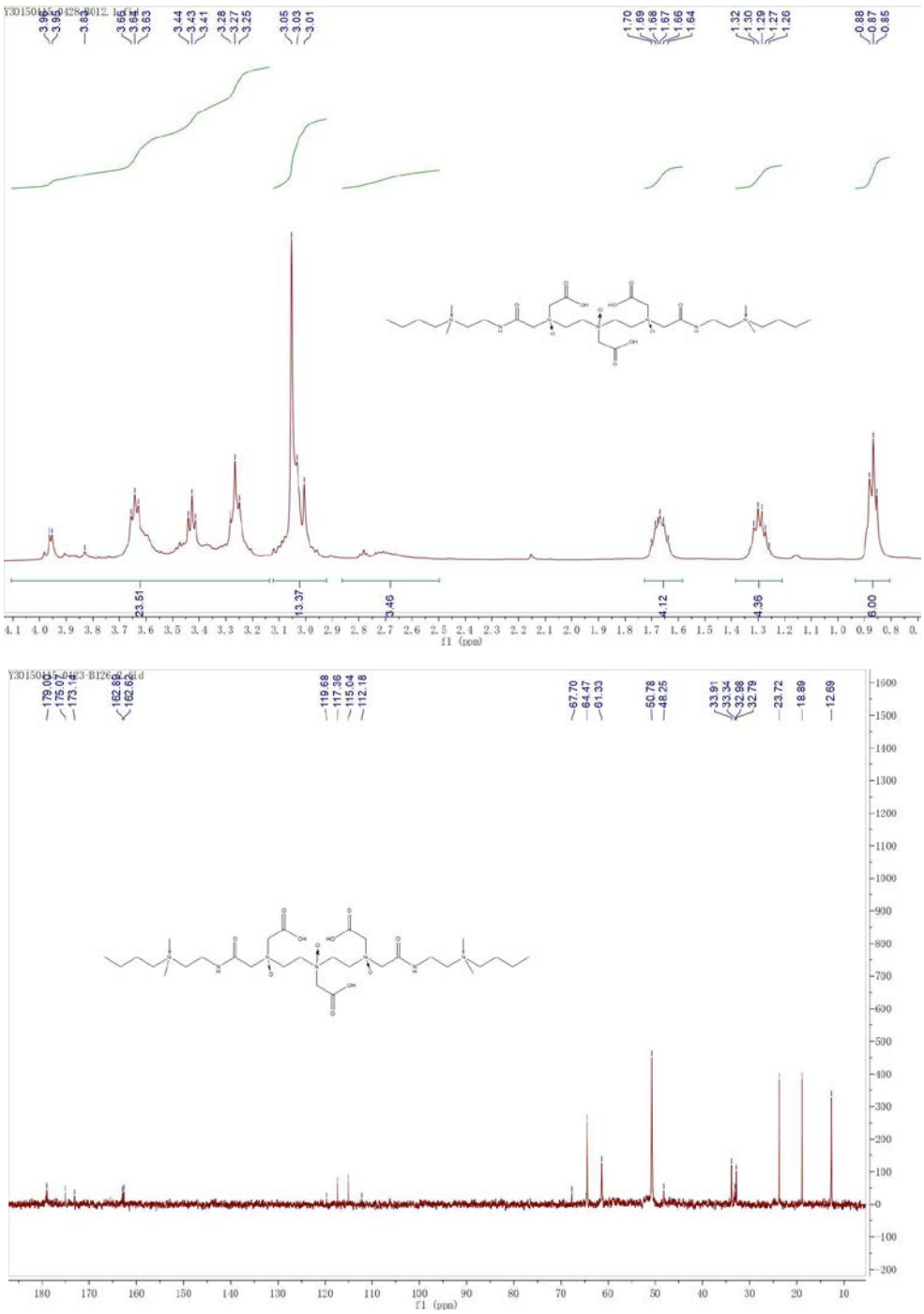

Fig. S5 ^{1}H NMR and ^{13}C NMR of spectra of compound **6**

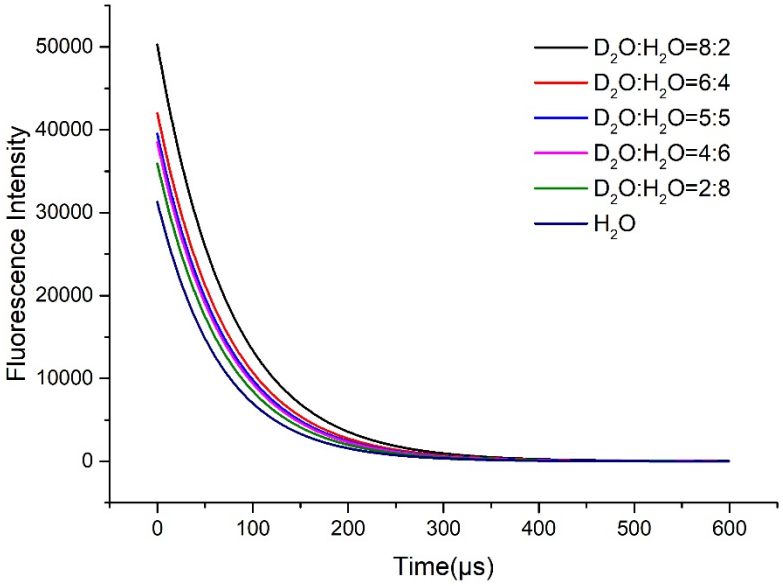

Fig. S6 Fluorescence decay of Eu (III) complex in different solvents.

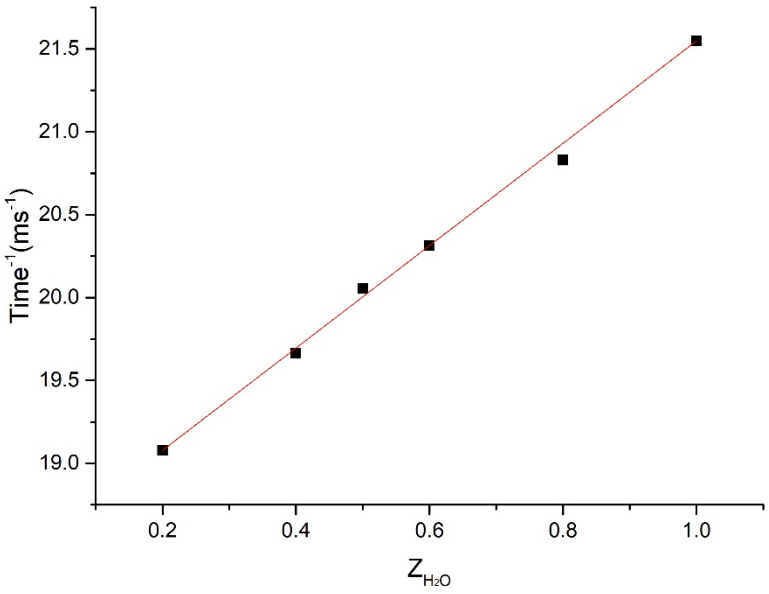

Fig. S7 Linear fitting of χ_{H2O} versus τ^{-1} of Eu (III) complex in different solvents.

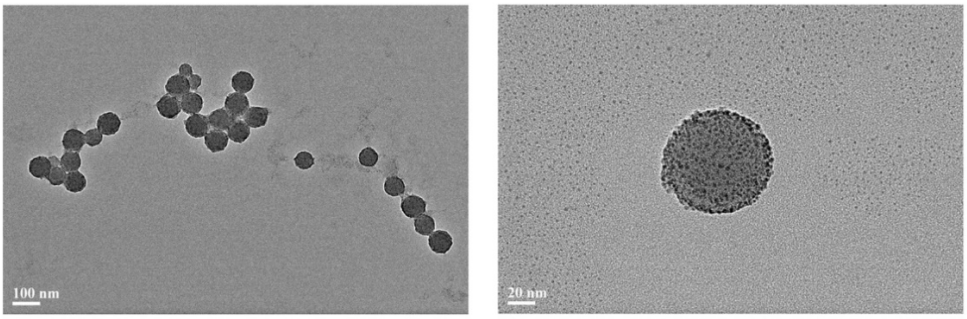

Fig. S8 TEM images of the initial SPB

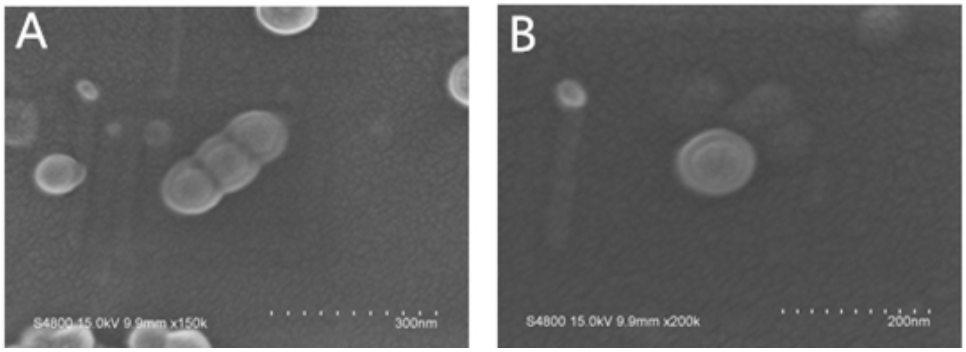

Fig. S9 SEM images of SPB-0.8

Fig. S10 Color photographs of xylenol orange solution[3] in the presence of 100 μM (a), 50 μM (b), 25 μM (c), 15 μM (d), 10 μM (e), 0 μM (f) of Gd(III) ion and 1 mM Gd-DTPA-NO-C_4 (g), respectively. The concentration of xylenol orange solution is 10 mg/L.

References:

1. M. Ballauff, *Prog. Polym. Sci.*, 2007, **32**, 1135-1151.
2. V. Montembault, J.-C. Soutif, J.-C. Brosse, F. Hilldré and J.-J. Le Jeune, *React. Funct. Polym.*, 1997, **32**, 43-52.
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