Supplementary Material (ESI) for Chemical Communications

High-Relaxivity MRI Contrast Agents Prepared from Miniemulsion Polymerization using Gadolinium(III)-Based Metallosurfactants

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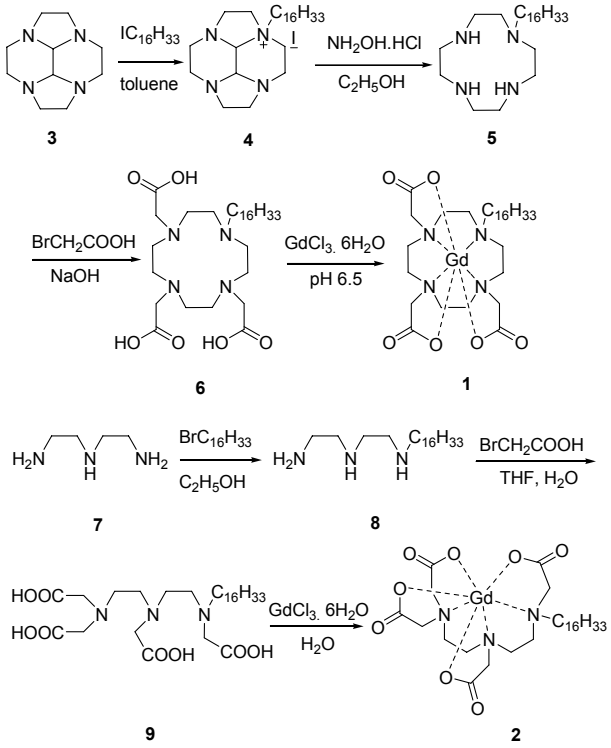
Materials: Toluene was distilled over sodium under nitrogen prior to use; Styrene (St) and divinylbenzene (DVB) were washed with an aqueous solution of sodium hydroxide (10 wt%), dried over anhydrous magnesium sulfate, distilled under reduced pressure and stored under nitrogen in a -10℃ refrigerator; Azobisisobutyronitrile (AIBN) was recrystallized from ethanol; 1-iodohexadecane was prepared from 1-bromohexadecane by reaction with sodium iodide in dry acetone. Other reagents are commercial grade and used without further purification.

Synthesis:



Scheme 1. Synthesis of Gd(III)-DO3A-based and Gd(III)-DTPA-based Metallosurfactants

(1RS,13SR,14RS)-1-Hexadecyl-4,7,10-triaza-1-azoniatetracyclo[5.5.2.0.$^{4.14}$0$^{10.13}$] tetradecane Iodide (4).[1] A solution of 1-iodohexadecane (3.22 g, 9.15 mmol) in toluene (10 ml) was slowly added to a solution of cyclen-glyoxal **3**[2] (1.46 g, 7.6 mmol) in toluene (10 ml). The mixture was stirred for 4-6 days at 60 □ under nitrogen. After solvent evaporation, the residue was extracted with diethyl ether for several times to remove the unreacted 1-iodohexadecane, and compound **4** was obtained as a yellow solid (3.13 g, 76%). ^{1}H NMR ($CDCl_3$) 0.88 (t, 3H), 1.25 (m, 22H), 1.35 (m, 2H), 1.44 (m, 2H), 1.81 (m, 2H), 2.39 (ddd, 1H), 2.68 (m, 1H), 2.71 (m, 1H), 2.81 (ddd, 1H), 2.83 (m, 1H), 2.86 (ddd, 1H), 3.04 (ddd, 1H), 3.20 (m, 3H), 3.29 (td, 1H), 3.41 (td, 1H), 3.45 (d, 1H), 3.75 (td, 1H), 3.8 (m, 3H), 4.1 (td, 1H), 4.22 (d, 1H), 4.5 (ddd, 1H).

1-Hexadecyl-1,4,7,10-tetraazacyclododecane (5).[3] Compound **4** (2.02 g, 5 mmol) was added to a

solution of $NH_2OH{\cdot}HCl$ (50 mmol) in dry ethanol (20 ml) and the mixture was refluxed for 2.5 h. After solvent evaporation, the solid was neutralized with 15% aqueous NaOH solution and extracted three times with dichloromethane. The organic layers were combined, dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The sticky solid obtained was then dissolved in ethanol (5 ml) and treated with concentrated HCl (5 ml). A white precipitate formed after a while, which was filtered and recrystallized in ethanol. The free amine product **5** was obtained by neutralizing the crystalline with 15% aqueous NaOH solution, and extracted with dichloromethane. Yield 1.15 g (79%). ^{1}H NMR ($CDCl_3$) 0.86 (t, 3H), 1.24 (m, 26H), 1.44 (quin, 2H), 2.42 (t, 2H), 2.55 (t, 4H), 2.60 (t, 4H), 2.65 (t, 4H), 2.80 (t, 4H).

1-Hexadecyl-1,4,7,10-tetraazacyclododecane-1,4,7-tri-acetic Acid (6).[1] Bromoacetic acid (1.26 g, 9 mmol) and NaOH (0.36 g, 9 mmol) were added to an aqueous solution of **5** (0.6 g, 1.5 mmol). The mixture was heated at 70 □, and the pH value was maintained between 10 and 12 by addition of 2 M NaOH until it did not change. The solution was then neutralized and cooled to room temperature and a white precipitate formed. The precipitate was collected by filtration and recrystallized in water to give **6** (0.48 g, 56%). ^{1}H NMR (D_2O) 0.89 (t, 3H), 1.18-1.45 (m, 26H), 1.80 (quin, 2H), 2.60-3.80 (m, 22H), 4.04 (s, 2H). MS (ESI+): m/z 571.6 ($M + H^+$).

[1-Hexadecyl-4,7,10-tris(carboxymethyl)tetraazacyclododecanato]gadolinium (1). Ligand **6** (0.2 g, 0.35 mmol) was dissolved in water (8 ml) at 50 □ and the pH value of the mixture was adjusted to 6.5. A solution of $GdCl_3.6H_2O$ (0.13 g, 0.35 mmol) in water (2 ml) was then slowly added. After completion of addition, the pH value of the mixture was adjusted back to 6.5 and the reaction mixture was stirred overnight at 50 □. The pH value was finally increased to 10 in order to precipitate all the unreacted $GdCl_3$. The mixture was filtered through a 0.2 μm Millipore filter and the filtrate was passed through an ionic exchange column (Amberlite 732). The collected fractions were freeze-dried to give **1** (0.16 g, 63%). MS (ESI+): m/z 726.3 ($M + H^+$).

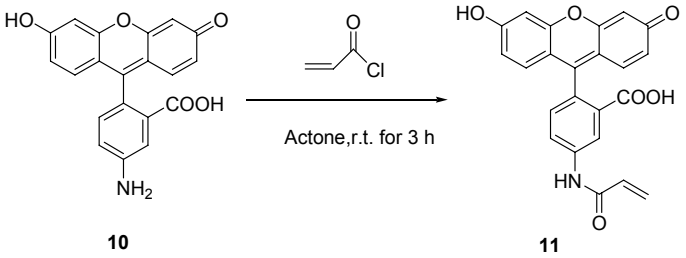
1-Hexadecyl-Diethylenetriamine (8).[4] A solution of 1-bromohexadecane (3.06 g, 10 mmol) in ethanol (10 ml) was added within 30 min to a stirred solution of diethylenetriamine **7** (10.32 g, 100 mmol) in ethanol (125 ml) at room temperature. NaOH (0.1 M) was then added to the reaction mixture until pH >7. The resulting solution was heated at 90□ for 6 h, stirred at room temperature for 18 h, and then the solvent was evaporated under reduced pressure. The residue was extracted with CH_2Cl_2 and brine, and the organic phase was collected and evaporated under reduced pressure. A white solid product was obtained (3.01 g, 92 %). ^{1}H NMR ($CDCl_3$): δ 2.80 (t, ^{3}J = 5.8 Hz, 2H), 2.73 (br, 4H), 2.68 (t, ^{3}J = 5.8 Hz, 2H), 2.59 (t, ^{3}J = 7.2 Hz, 2H), 1.41(m, 2H), 1.25 (br, 26H), 0.88 (t, ^{3}J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 52.5, 50.1, 49.6, 49.4, 41.8, 31.9, 30.2, 29.7, 27.4, 22.7, 14.1.

1-Hexadecyl-Diethylenetriamine tetraacetic acid (9). To a solution of **8** (0.33 g, 1 mmol) in THF (25 ml), bromoacetic acid (1.39 g, 10 mmol) in H_2O (10 ml) was added over 30 min. The pH value of the reaction mixture was maintained at 10 by constant addition of NaOH (2 M). After addition, the mixture was refluxed for 6 h, and then treated with HCl until the pH value of the solution reached 1. All the volatile components were removed under reduced pressure. The residue was washed for several times with cold ethanol and dissolved again in H_2O (pH 8) and treated with HCl until precipitate formed. The white solid was filtered, washed with cold H_2O, and dried in a vacuum desiccators (0.29 g, 67 %). ^{1}H NMR (D_2O): δ 3.11 (br, 8H), 2.64 (br, 4H), 2.57 (m, 4H), 2.49 (m, 2H), 1.39 (br, 4H), 1.22 (br, 24H), 0.79 (t, 3H). ^{13}C NMR (CD_3OD/ $CDCl_3$) δ 59.7, 49.0, 31.5, 29.2, 28.9, 22.2, 13.4.

[1-Hexadecyl-Diethylenetriamine tetraacetato] gadolinium (2). Ligand **9** (0.28 g, 0.5 mmol) was dissolved in water (10 ml) at 70 □ and the pH value of the solution was adjusted to 8. A solution of $GdCl_3.6H_2O$ (0.18 g, 0.5 mmol) in water (1 ml) was slowly added. The pH value of the reaction mixture was maintained at 9 by addition of NaOH (2 M). After stirred at 70 □ overnight, the mixture was filtered through a 0.2 μm Millipore filter. The collected filtrate was evaporated under reduced pressure to give the complex **2** as a white solid (0.13 g, 36 %). MS (ESI+) 717.4 ($M + H^+$).

Typical preparation of Gd(III)-polymer nanoparticles through miniemulsion polymerization. A monomer solution containing styrene (160 mg), DVB (40 mg), cetyl alcohol (CA, 6 mg) and AIBN (7 mg)

was added to the continuous phase consisting of 18 mg compound **1** (or **2**) and 25 mg Tween 60 dissolved in 6 g of water. The mixture was stirred for 30 min and then ultrasonicated for 4-12 min (4, 8, 12 min for 56, 48, 35 nm **1**-polymer particles, respectively) at a power of 180 W. The resultant miniemulsion was purged with N_2 for 20 min and then the polymerization was carried out at 75 □ for 6 h.



10 **11**

Scheme 2. Synthesis of 5-Acrylamidofluorescein

5-Acrylamidofluorescein (11).[5] 5-Aminofluorescein (**10**) (0.68 g, 2.0 mmol) was suspended in dry acetone (25 ml) in a 50 ml round-bottom flask along with a magnetic stir bar. The dark orange suspension was submersed in an ice bath and stirred for 15 min. Acryloyl chloride (0.18 ml, 2.2 mmol) in dry acetone (0.5 ml) was slowly added to the suspension in 10 min. The mixture was stirred for 3 h at room temperature. During this period, yellow crystals formed. The yellow crystals were then separated from the solution via filtration and washed with cold acetone and diethyl ether. The final product was obtained as an orange powder by chromatography on a neutral alumina column using dichloromethane: ethanol (10 : 1) as an eluent (0.50 g, 60%). ^{1}H NMR (DMSO-d6): 5.83 (dd, 1H), 6.32 (dd, 1H), 6.44-6.67 (m, 1H), 7.22 (d, 1H), 7.88 (dd, 1H), 8.40 (d, 1H), 10.10 (s, 2H), 10.60 (s, 1H).

Preparation of fluorescent colloids through miniemulsion polymerization. A mixture containing styrene (160 mg), 5-AF (3 mg), CA (2 mg) and AIBN (7 mg) was added to a suspension of **1** (18 mg) and Tween 80 (25 mg) in water (6 g). The mixture was stirred for 30 min and then ultrasonicated for 6 min at a power of 180 W. The resultant miniemulsion was purged with nitrogen for 20 min and then the polymerization was carried out at 75 □ for 6 h.

Characterization:

NMR. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on an Ultra Shield 400 spectrometer (Bruker BioSpin AG, Magnet System 400 MHz/54mm) in deuterated solvents.

XPS. XPS experiments were carried out on a RBD upgraded PHI-5000C ESCA system (Perkin Elmer) with Mg Kα radiation (hν=1253.6 eV) or Al Kα radiation (hν=1486.6 eV). The whole spectra (0~1100 eV) and the narrow spectra of all the elements with much high resolution were both recorded by using RBD 147 interface (RBD Enterprises, USA) through the AugerScan 3.21 software. Binding energies were calibrated by using the containment carbon (C_{1s} = 284.6 eV). The data analysis was carried out by using the RBD AugerScan 3.21 software provided by RBD Enterprises or XPSPeak4.1 provided by Raymund W. M. Kwok (The Chinese University of Hongkong, China).

ICP-AES. The contents of Gd were measured through inductively coupled plasma atomic emission spectrometry (ICP-AES, IRIS 1000).

MS. Mass spectra were obtained with a Micromass LCTTM mass spectrometer using ESI method.

Dynamic Light Scattering (DLS). The sizes and size distributions of colloids were measured using a Zeta Sizer nano series laser light scattering system (Malvern Instrument Corporation). Scattered light was detected at an angle of 90° at 25 □ in a quartz cell. For sample preparation, the original colloids suspension was diluted to 1wt. % in deionized water. The data for each sample were obtained in three independent measurements.

Transmission Electron Microscopy (TEM). TEM images were obtained on a JEOL JSM-2010 at an acceleration voltage of 100 kV. TEM samples were prepared by depositing a drop of dilute colloids on a carbon/copper grid.

Scanning Electron Microscopy (SEM). The morphology of colloids was observed on a HITACHI S-4800 SEM. The samples were prepared by depositing a drop of dilute colloids on an aluminum foil, dried under room temperature and then sputter-coated with gold for 60 s.

Fluorescence Spectroscopy. Fluorescence spectra were recorded with a Fluorolog-3-p spectrophotometer. The split widths were 3 nm for both excitation and emission. The samples were excited at the wavelength of 468 nm.

Magnetic Resonance Imaging (MRI). MRI was performed on a 3T Siemens MAGNETOM Trio, a TIM System and a 12-channel head coil (Siemens Medical Solutions, Erlangen, Germany). Parameters for T_1 weighted images were TI = 900 ms and TE = 11 ms. The samples were prepared at ten to twelve different concentrations by diluting the original colloids in 1.5 ml centrifuge tubes.

T_1 Relaxivity Measurements. T_1 Relaxivity Measurements were also performed on a 3T Siemens MAGNETOM Trio, a TIM System and a 12-channel head coil (Siemens Medical Solutions, Erlangen, Germany), at ambient temperature. The samples were diluted to different concentrations in 1.5 ml centrifuge tubes. For T_1 measurement, the samples were imaged collectively with a high-resolution inversion recovery pulse sequence (TR/TE = 11ms, TI= 24 100 200 400 600 900 1200 2000 3000 5000 ms. FOV = 150 mm × 150 mm, Matrix = 320 × 320). The resulting images were analyzed on a pixel-by-pixel basis to a single exponential. These T_1 values were averaged over at least 45 pixels in the center of each sample and plotted as $1/T_1$ VS $[Gd^{3+}]$. The slope of this line was the millimolar relaxivity, r_1.

CMC Measurements. CMC Measurements *via* r1 plotting were also performed on a 3T Siemens MAGNETOM Trio, a TIM System and a 12-channel head coil (Siemens Medical Solutions, Erlangen, Germany), at ambient temperature. The samples were prepared in different concentrations in 1.5 ml centrifuge tubes. $1/T_1$ values obtained were plotted vs concentration of metallo-surfactants. When the concentration is over CMC, metallo-surfactants aggregate in micelles, which have higher relaxivity due to slower tumbling rate. Thus, the cross-point of two linear fits at low and high concentrations is CMC.

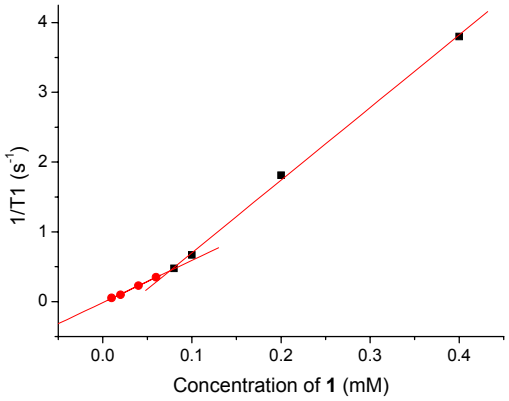


Figure S1. CMC determination of **1** via r1 plotting. r1 = 6.0/10.4 $mM^{-1}s^{-1}$, CMC = 0.076 mM.

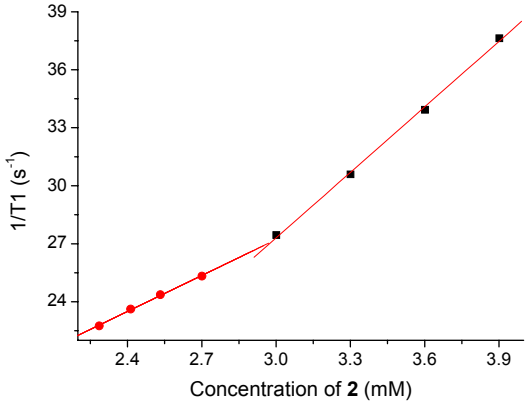


Figure S2. CMC determination of **2** via r1 plotting. r1 = 6.2/11.0 $mM^{-1}s^{-1}$, CMC = 3.0 mM.

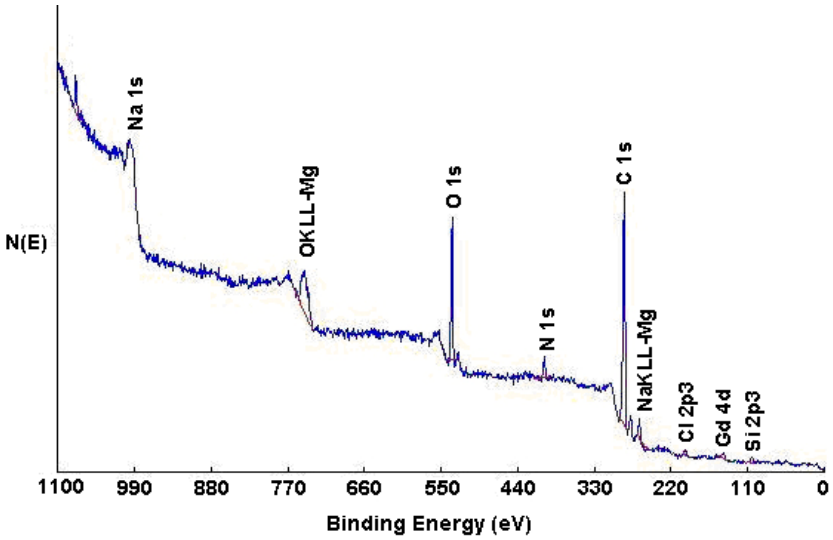


Figure S3. XPS spectrum of metallo-colloids

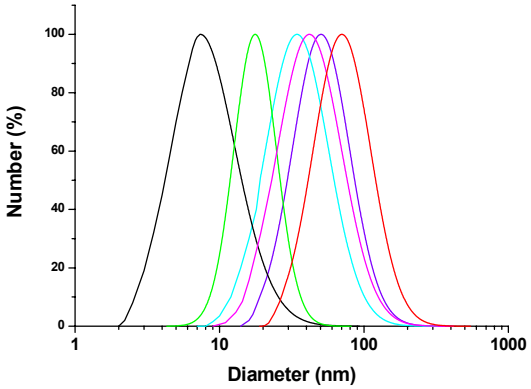


Figure S4. Representative DLS curves of metallo-colloids with varied sizes in water. (black: colloid-**1**, D_h = 7.4 nm, r_1 = 20.4 $mM^{-1}s^{-1}$; green: colloid-**1**, D_h = 19.9 nm, r_1 = 11.1 $mM^{-1}s^{-1}$; cyan: colloid-**1**, D_h = 34.8 nm, r_1 = 7.5 $mM^{-1}s^{-1}$; magenta: colloid-**1**, D_h = 47.5 nm, r_1 = 8.8 $mM^{-1}s^{-1}$; violet: colloid-**2**, D_h = 56.0 nm, r_1 = 9.1 $mM^{-1}s^{-1}$; red: colloid-**2**, D_h = 78.1 nm, r_1 = 8.9 $mM^{-1}s^{-1}$).

There is no simple relationship between sizes of colloids and relaxivities, which is similar to a micelle system recently reported.[6]

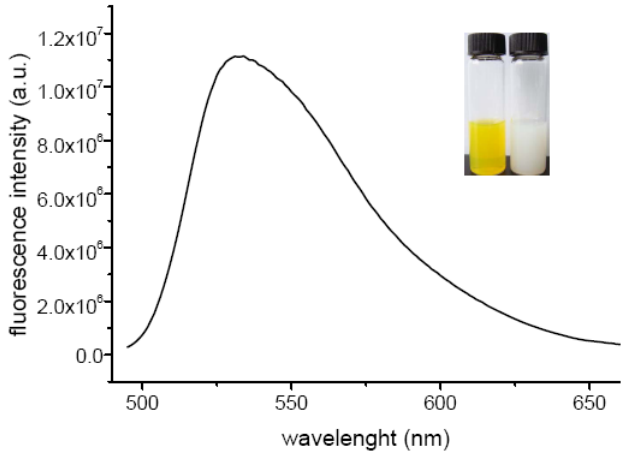


Figure S5. Emission spectra (Ex = 468 nm) of fluorescent colloids in water (inset shows photos of metallo-colloids with (left) and without (right) 5-AF)

References:

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