Supporting information

Micelles Based on Biodegradable Poly(L-glutamic acid)-*b*-Polylactide with Paramagnetic Gd Ions Chelated to the Shell Layer as a Potential Nanoscale MRI-Visible Delivery System

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Synthesis of *p*-(s-2-[4-Aminobenzyl]-diethylenetriamine penta-tert-butyl acetate) (NH_2-Bz-DTPA[t-butyl ester])

Materials and Methods. (S)-p-Nitrophenylalanine was purchased from BACHEM. 1 M borane-THF, thionyl chloride, ethylene diamine, tert-butyl bromoacetate, and 10% palladium on carbon were purchased from Acros. Hydrogenation apparatus was obtained from Parr Instrument Co. (Moline, IL). The mass spectra were obtained using an Agilent 1100 LC-MSD trap system VL instrument.

Synthetic Procedures. The reaction scheme for the synthesis of NH_2-Bz-DTPA[t-butyl ester] is shown in **figure 1**. NH_2-Bz-DTPA[t-butyl ester] was synthesized in five steps according to previously reported procedures, with one modification [1, 2]. Instead of using hydrogen chloride gas in the first step in the preparation of methyl 4-nitrophenylalaninate hydrochloride gas, we used thionyl chloride, which as a liquid is much easier to handle.

Methyl (S)-p-Nitrophenylalanate (1). To a stirred anhydrous methanol solution (60 mL) of thionyl chloride (5 mL, 8.18 g, 0.0687 mol) in an ice bath was added 4-nitrophenylalanine (12.0 g, 0.0526 mol) dropwise, and the reaction mixture was stirred at room temperature for about 20 h. Removal of the solvent under reduced pressure on a rotary evaporator yielded 14.0 g (97%) compound **1** as a white solid. ^{1}H NMR (D_2O) δ (ppm): 3.29 (dd, 1H, J = 14.4, 7.2 Hz), 3.39 (dd,

1H, J = 14.2, 6.3 Hz), 3.74 (s, 3H), 4.43 (t, 1H, J = 13.8 Hz), 7.44 (d, 2H, J= 8.4 Hz), 8.17 (d, 2H, J = 8.7 Hz).

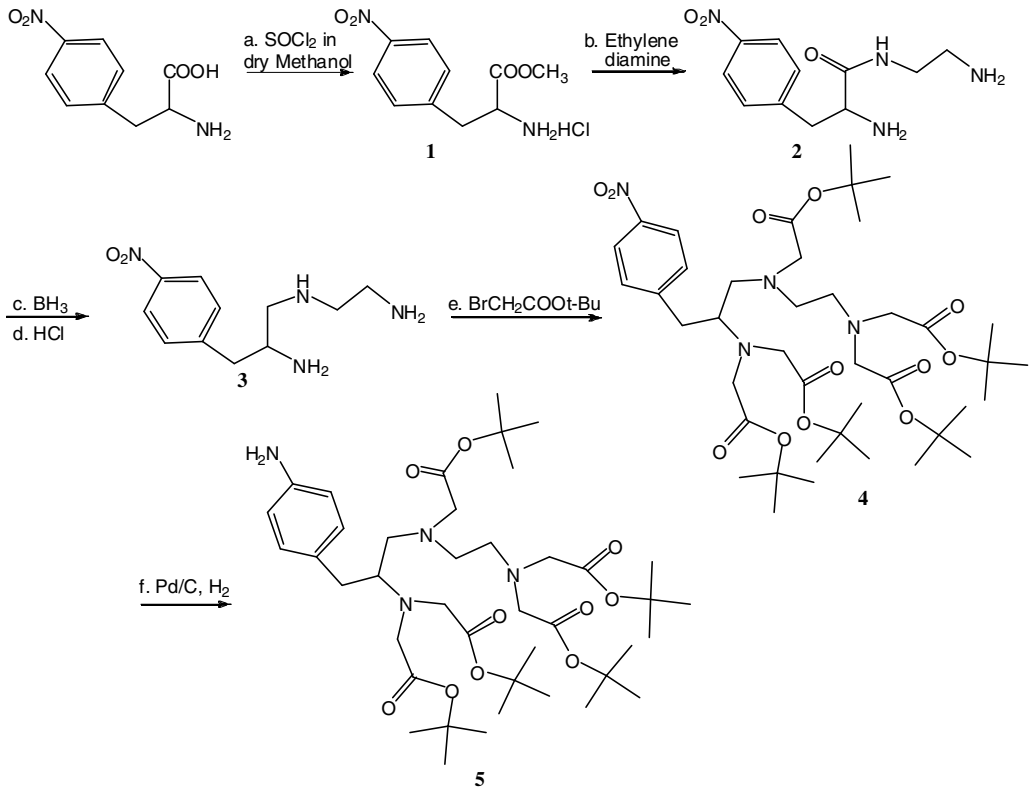

figure 1 Synthetic scheme for NH_2-Bz-DTPA[t-butyl ester]

(S)-p-Nitrophenylalanine N-(2-Aminoethyl) Amide (2). To stirred ethylenediamine (13.5 mL, 12.14 g, 0.20 mol) was slowly added methyl (S)-p-Nitrophenylalanate (4.5 g, 0.0173 mol) via a powder funnel at room temperature under Ar over 2h. After 24 h, the reaction solution was concentrated under reduced pressure to a brown oil. To the resulted oil was added 7M ammonium solution (31 mL), and the pH was adjusted to 11.5 with a 50% NaOH solution. The aqueous phase was extracted with ten 35 mL portions of CH_2Cl_2. The combined organic extract was dried over Na_2SO_4, filtered, concentrated, and dried under reduced pressure to afford 3.0 g (68.9%) of a yellow solid compound **2**. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.45(br s, 4H), 2.80 (t, 2H, J =

6.2 Hz), 2.95(dd, 1H, J = 8.7, 13.8Hz), 3.33 (m, 3H), 3.69 (dd, 1H, J = 4.5, 8.7 Hz), 7.40 (d, 2H, J = 9.0 Hz), 7.52 (br s, 1H), 8.20 (d, 2H, J = 9.0 Hz).

1-[(4-Nirophenyl) methyl]diethylenetriamine (3). To a stirred anhydrous THF solution (100mL) of (S)-p-Nitrophenylalanine N-(2-Aminoethyl) Amide (3.0 g, 0.0119 mol) was added dropwise a 1M BH_3/THF (100 mL) solution in a cooling bath (5–10 °C) under Ar. After the addition is finished, the reaction mixture was gradually warmed and heated to reflux for 15 h. The reaction solution was cooled to 5 °C, and water was added dropwise until the evolution of gas ceased. The reaction mixture was concentrated under reduced pressure to a yellow residue. 70 mL of HCl (6M) was added to the residue, refluxed for 3 h and stirred at room temperature for 24h. The reaction solution was concentrated under reduced pressure, and the residue was dissolved in 100 mL ~14% ammonium and the pH was adjusted to 11.5 with 5 M KOH. The reaction solution was extracted with $CHCl_3$ (6 × 30 mL), and the combined $CHCl_3$ extract was dried over Na_2SO_4, filtered, concentrated and dried under reduced pressure to yield compound **3** as a hygroscopic solid (2.5 g, 88.3 %). ^{1}H NMR ($CDCl_3$) δ (ppm):1.49(br s, 5H), 2.43(dd, 1H, J = 4.8, 12.0 Hz), 2.55-2.76 (m, 6H), 2.84 (dd, 1H, J = 4.8, 13.5 Hz), 3.05-3.09 (m, 1H), 7.33 (dd, 2H, J = 7.5 Hz), 8.08 (dd, 2H, J = 7.5 Hz).

1-(S)-(p-Nitrobenzyl)diethylenetriaminepentaacetic Acid Penta-t-butyl ester (4). To an anhydrous DMF solution (94 mL) of a mixture of compound **3** (2.5 g, 0.01 mol) and DIPEA (21.9 mL) was added *t*-butyl bromoacetate (14 mL, 18.49 g, 0.0948 mol), and then KI (1.92 g, 0.012 mol) was added in one portion. The reaction mixture was stirred at room temperature under Ar for ~ 18 h, and concentrated to dryness under reduced pressure using a water bath with temperature below 35 °C. The residual was extracted with 130 mL diethyl ether/65 mL water, and the organic phase was washed with water (65 mL), a saturated NaCl solution (33mL). The organic layer was dried over Na_2SO_4, filtered and evaporated to dryness, and the residue was chromatographed on silica gel with 25% ethyl acetate in hexane as the eluent to provide 6.8 g

(84.1%) of **4** as a yellow oil. ^{1}H NMR ($CDCl_3$) δ (ppm):1.35-1.51(m, 45H), 2.42(dd, 1H, J = 8.4, 13.5 Hz), 2.70-3.11 (m, 8H), 3.27 (s, 2H), 3.30-3.71 (m, 8H), 7.45 (d, 2H, J = 8.7 Hz), 8.07 (d, 2H, J = 8.7 Hz).

Penta-t-Butyl 1-(S)-(p-Aminobenzyl)-diethylenetriamine-pentaacetate (5). To a stirred solution of 6.2 g (7.66 mmol) of **4** in 50 mL of methanol was added 1.0 g of 10% Palladium on carbon under Ar. The hydrogenation reaction was underwent using a hydrogenation apparatus. After 24 h, the reaction was completed, monitored by TLC (eluent Hexane/EtOAc 1:2). The reaction solution was filtered through a pad of pre-washed Celite, and the pad was washed with methanol until no product was detected. The filtrate was evaporated under reduced pressure to dryness, and the residue was chromatographed on silica gel with 15% methanol in chloroform as eluent to give 5.2 g (87.1%) of **5** as a yellow oil. ^{1}H NMR (DMSO-d6) δ (ppm): 1.42-1.47(m, 45H), 2.48-2.55(m, 2H), 2.72-2.79(m, 6H), 3.02-3.04(m, 1H), 3.37-3.54(m, 10H), 6.60(d, J=8.4 Hz, 2H), 7.01(d, J=8.4 Hz, 2H). ESI-MS: Calcd. for $(M+H)^+$ $C_{41}H_{71}N_4O_{10}$: 779.52; Found: 779.52.

Reference

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