Furry Nanoparticles: Synthesis and Characterization of Nanoemulsion-Mediated Core Crosslinked Nanoparticles and Their Robust Stability *in vivo*

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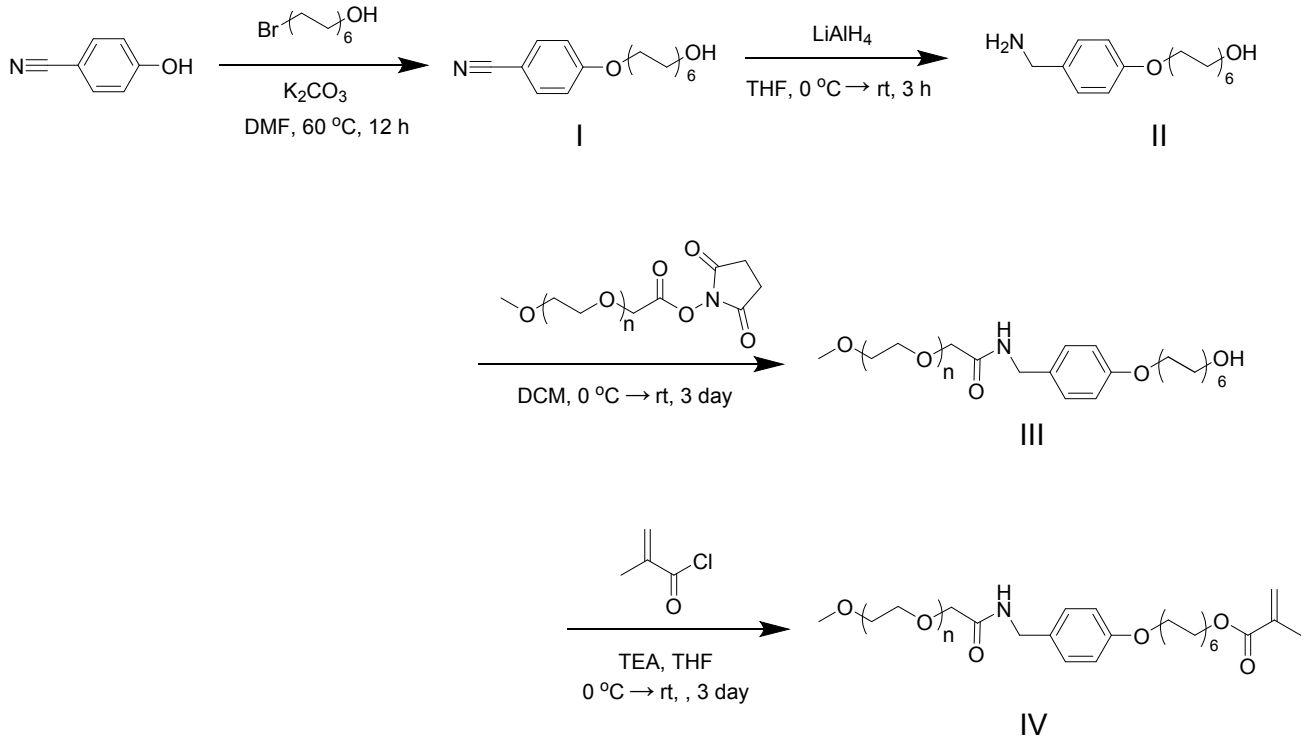
Synthesis procedure



Scheme S1. Synthesis scheme of compound IV ($PEG_{2k}C_{12}MA$)

Synthesis of compound I. 4-hydroxybenzonitrile (2.01 g, 16.9 mmol), 12-Bromo-1-dodecanol (4.05 g, 15.3 mmol), and potassium carbonate (6.33 g, 45.8 mmol) were dissolved in dry dimethylformamide (DMF) (10 mL). The reaction mixture was stirred for 12 h at 60 °C. The mixture was cooled to room temperature, and then the reaction was quenched with water (10mL). The mixture was washed with saturated solution of sodium chloride. The organics was dried over Magnesium sulfate ($MgSO_4$) and concentrated in vacuo. The crude product was purified by column chromatography eluted with dichloromethane (DCM)/ethyl acetate (EtOAc) (10:1), which afforded a white powder (yield: 4.30 g, 14.2 mmol, 83%). ^{1}H NMR (500 MHz, Chloroform-d): δ (ppm) = 7.57 (d, J = 10 Hz, 2H), 6.93 (d, J = 10 Hz, 2H), 3.99 (t, J = 5 Hz, 2H), 3.64 (q, J = 5.0 Hz, 2H), 1.80 (m, 2H), 1.57 (m, 2H), 1.48-1.24

(m, 16H). ^{13}C NMR (125 MHz, CDCl3): δ (ppm) = 162.43, 133.92, 119.32, 115.14, 103.56, 68.37, 63.02, 32.75, 29.55, 29.51, 29.48, 29.38, 29.27, 28.93, 25.89, 25.71. Anal. Calcd for (C19H29NO2): C 75.21, H 9.63, N 4.62. Found: C 75.07, H 9.61, N 4.92.

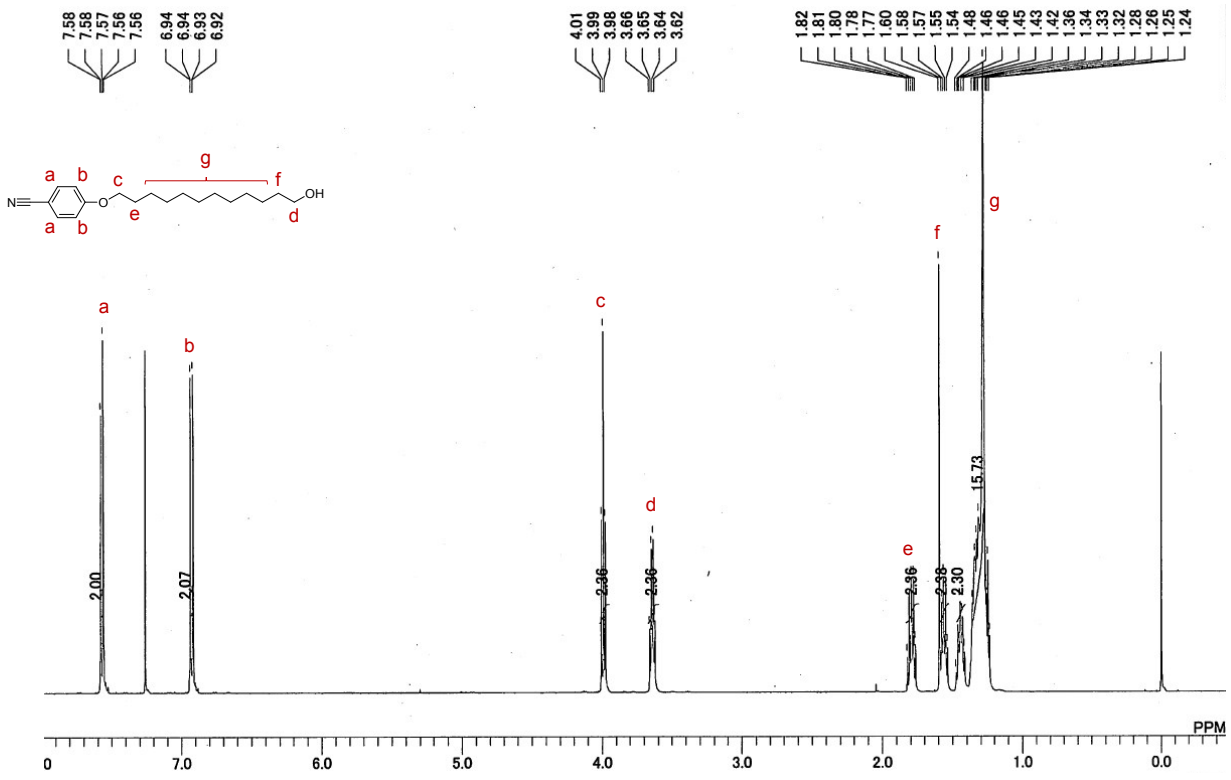
Synthesis of compound II. Compound I (4.30 g, 14.2 mmol) was dissolved in dry tetrahydrofuran (THF), then the solution was added into a flask containing lithium aluminium hydride (3.23 g, 85.1 mmol) under nitrogen atmosphere at 0 °C. The reaction mixture was stirred for 3 h at room temperature. The reaction was quenched with water (3.0 mL), then added 15 wt% NaOH aqueous solution (3.0 mL), giving a precipitation. The precipitation was filtered and washed with DCM, and then concentrated in vacuo. The crude product was purified by column chromatography eluted with DCM/EtOAc (10:1) (1:3), which afforded a yellow powder (yield: 3.50 g, 11.4 mmol, 80%). ^{1}H NMR (500 MHz, Chloroform-d): δ (ppm) = 7.21 (d, J = 10 Hz, 2H), 6.86 (d, J = 10 Hz, 2H), 3.94 (t, J = 5 Hz, 2H), 3.8 (s, 2H), 3.63 (q, J = 5.0 Hz, 2H), 1.77 (m, 2H), 1.55 (m, 2H), 1.46-1.28 (m, 16H). ^{13}C NMR (125 MHz, CDCl3): δ (ppm) = 158.06, 135.14, 128.22, 114.52, 68.02, 62.92, 45.84, 32.79, 29.55, 29.50, 29.40, 29.31, 29.24, 25.98, 25.72. Anal. Calcd for (C19H33NO2): C 74.22, H 10.82, N 4.58. Found: C 73.51, H 10.53, N 4.64. ESI–MS (M+Na): calcd for $C_{19}H_{33}NNaO_2^+$ 330.24, found 330.2.

Synthesis of compound III. PEG (2000 g/mol) bearing N-hydroxylsuccinimide (0.504 g, 0.252 mmol) and triethylamine (0.0506 g, 0.500 mmol) were dissolved in dry DCM. The compound II (0.117 g, 0.381 mmol) dissolved in dry DCM was added into the reaction mixture at room temperature. The reaction mixture was stirred for 48 h at room temperature, and then concentrated in vacuo. The crude

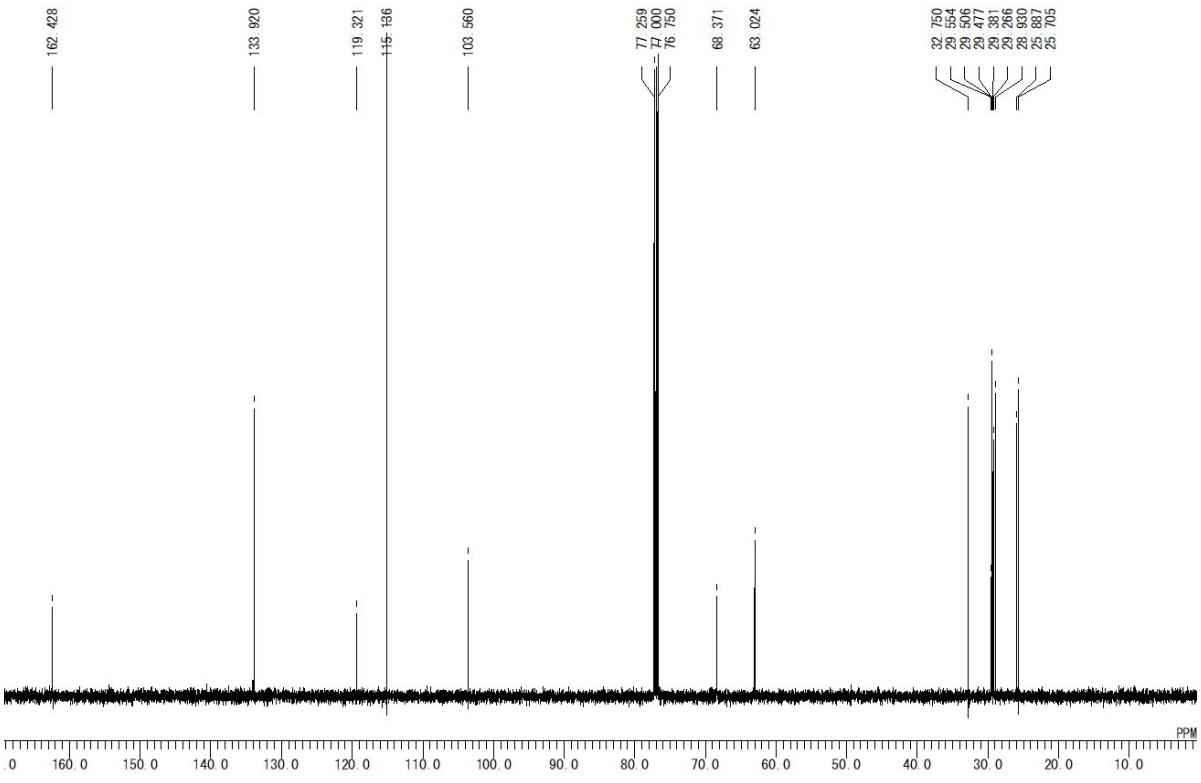
product was dissolved in DCM, and then precipitated into diethyl ether, which afforded a white powder (yield: 0.465 g, 0.232 mmol, 92%). ^{1}H NMR (500 MHz, Chloroform-d): δ (ppm) = 7.35 (s, 1H), 7.21 (d, J = 10 Hz, 2H), 6.84 (d, J = 10 Hz, 2H), 4.40 (s, 2H), 4.04 (s, 2H), 3.93 (t, J = 5 Hz, 2H), 3.8-3.3 (m, 181H), 1.77 (m, 2H), 1.55 (m, 2H), 1.46-1.28 (m, 16H).

Synthesis of compound IV ($PEG_{2k}C_{12}MA$). The compound III (1.05 g, 0.477 mmol) and triethylamine (0.924 g, 9.13 mmol) were dissolved in drty THF. Methacryloyl chloride (0.954 g, 9.13 mmol) dissolved in dry THF was drop wised into the reaction mixture at 0°C. The reaction mixture was stirred for 24 h at room temperature, and then concentrated in vacuo. The crude product was dissolved in DCM, and then precipitated into diethyl ether, which afforded a white powder. The powder was then dissolved in water, then purified by dialysis with (Spectrapor; cutoff 3.5 kDa) for 3 days. (yield: 0.703 g, 0.311 mmol, 69%). ^{1}H NMR (500 MHz, Chloroform-d): δ (ppm) = 7.35 (s, 1H), 7.22 (d, J = 10 Hz, 2H), 6.84 (d, J = 10 Hz, 2H), 6.10 (s, 1H), 5.55 (s, 1H), 4.4 (s, 2H), 4.04 (s, 2H), 3.93 (t, J = 5 Hz, 2H), 3.8-3.3 (m, 181H), 1.94 (s, 3H), 1.80 (m, 2H), 1.67 (m, 2H), 1.50-1.22 (m, 16H).

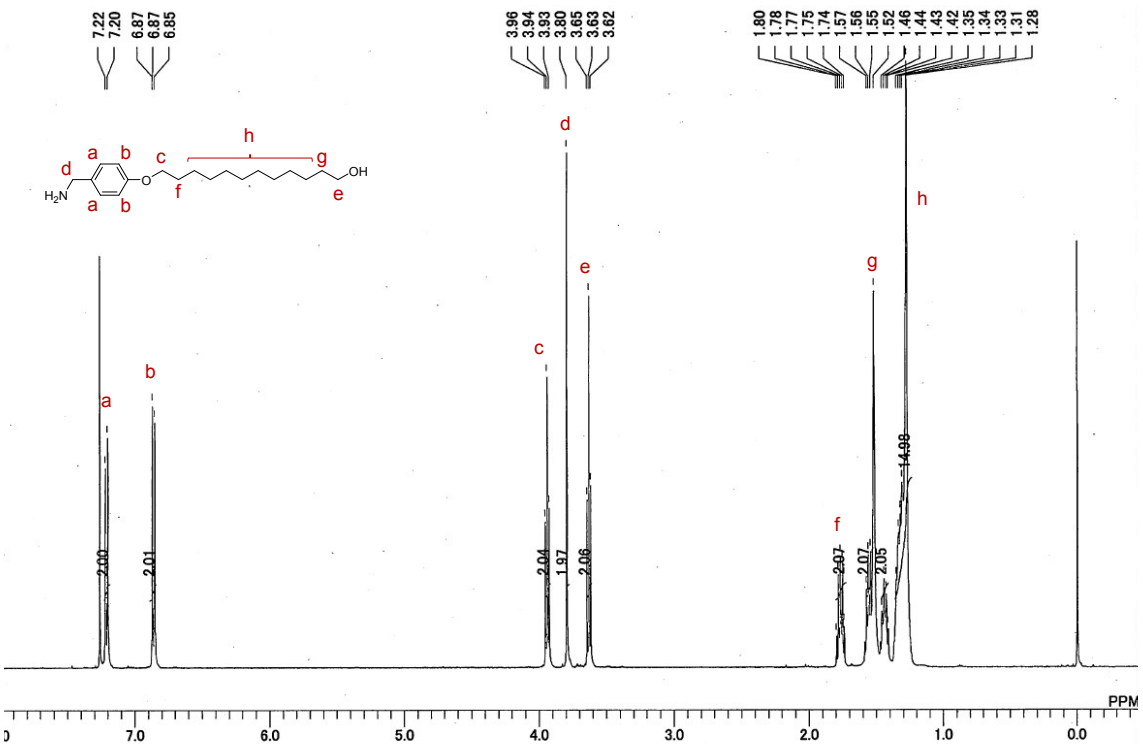
NMR spectra



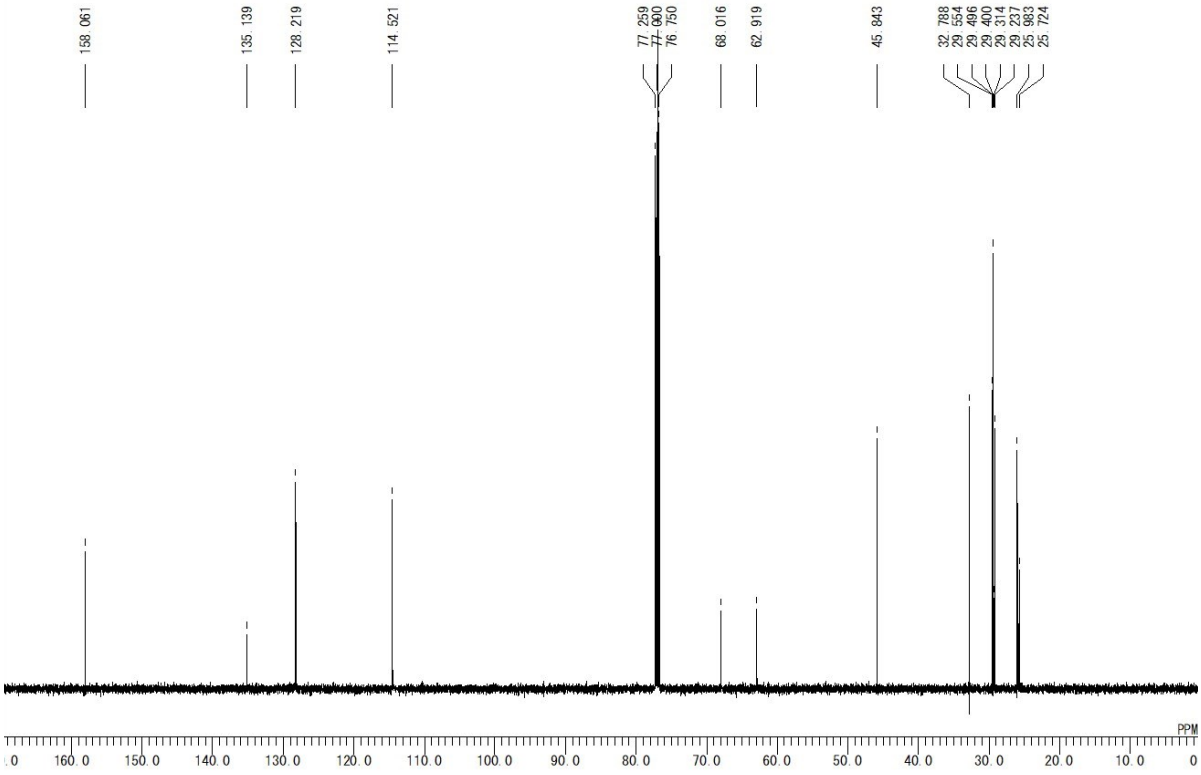
^{1}H-NMR spectrum of compound I.



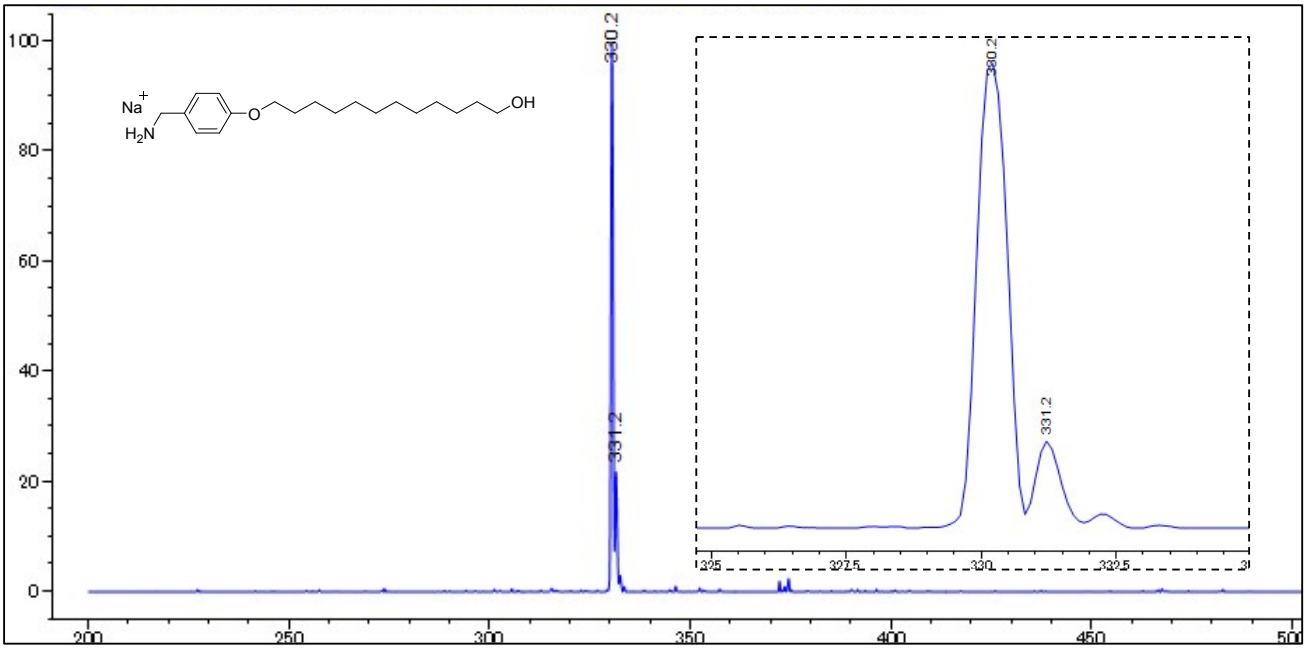
^{13}C-NMR spectrum of compound I.



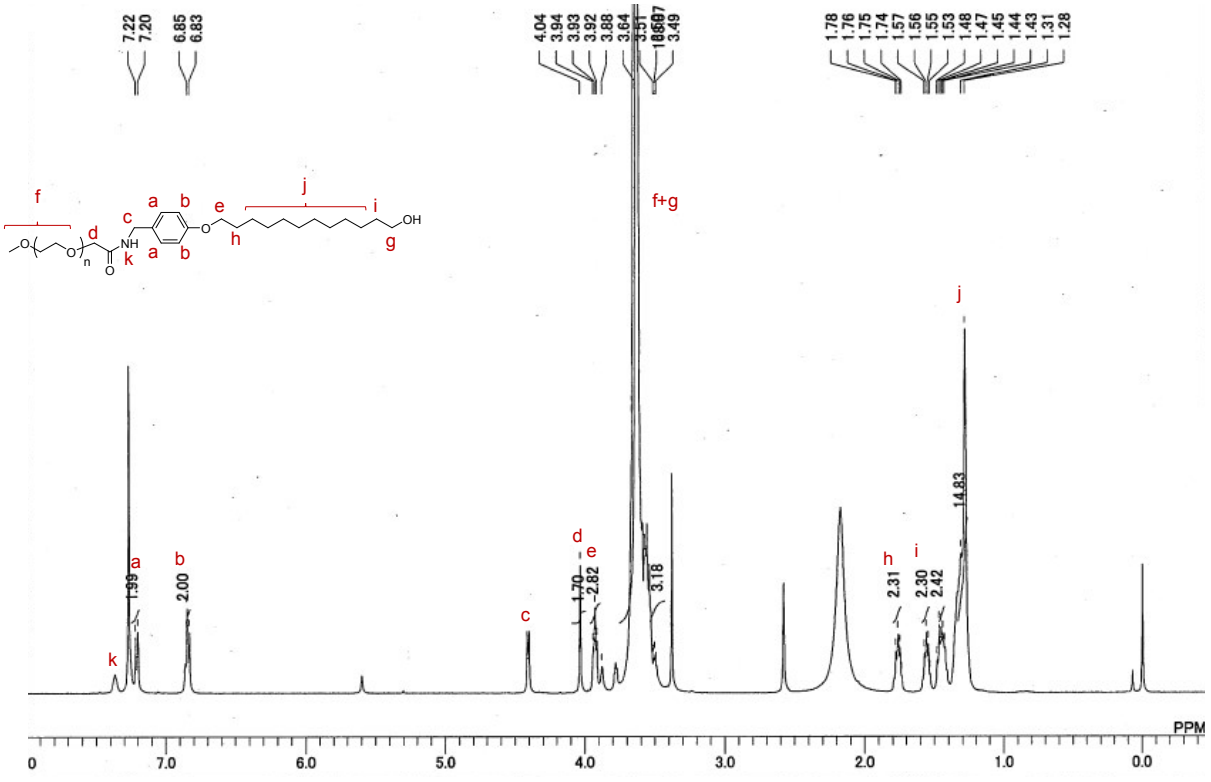
^{1}H-NMR spectrum of compound II.



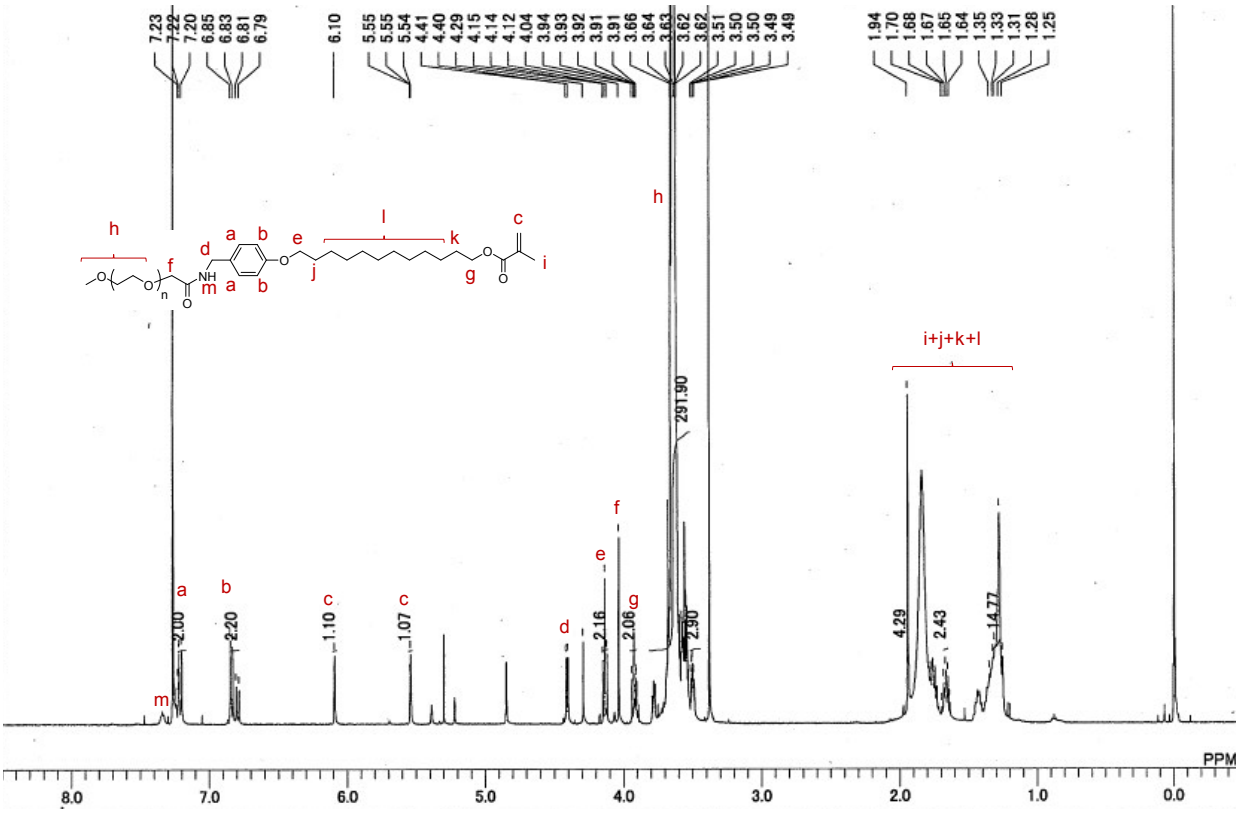
^{13}C-NMR spectrum of compound II.



Mass spectrum of compound II



^{1}H-NMR spectrum of compound III.



^{1}H-NMR spectrum of compound IV.

Table S1

Table S1. Concentration dependence of refractive index increment for $PEG_{2k}C_{12}MA$ micelles and f-NPs in 150 mM aqueous NaCl.

φ	dn/dc
0	0.139
2	0.241
5	0.240
7	0.239
10	0.238
12	0.238

Figure S1

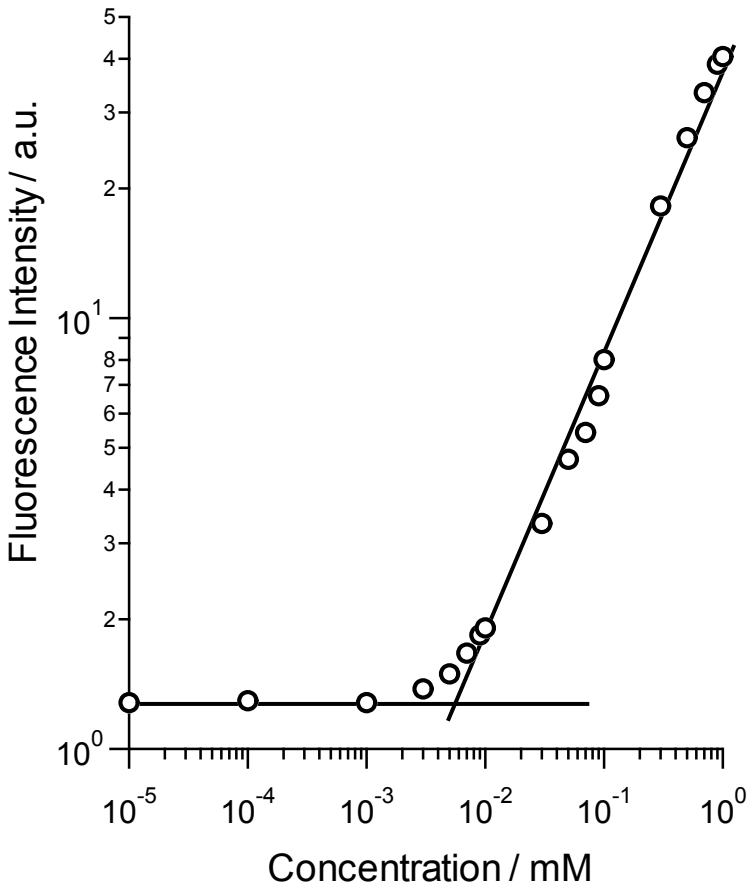


Figure S1. The fluorescence intensity of the ANS spectra at 480 nm plotted against the concentration of $PEG_{2k}C_{12}MA$ in 150 mM aqueous NaCl.

Figure S2

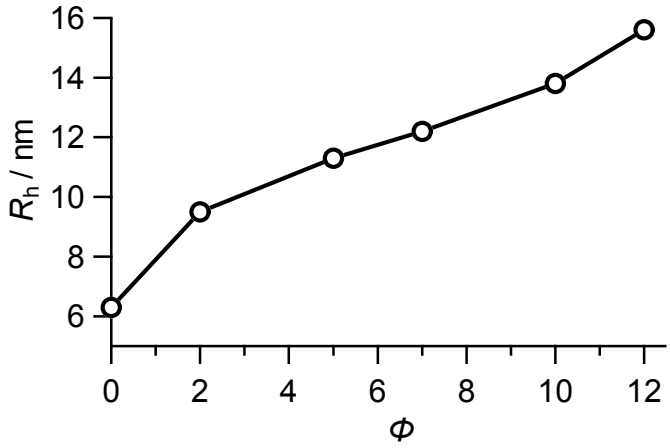


Figure S2. R_h plotted against φ {= [DVB]/[$PEG_{2k}C_{12}MA$]} determined by DLS measurements.

Figure S3

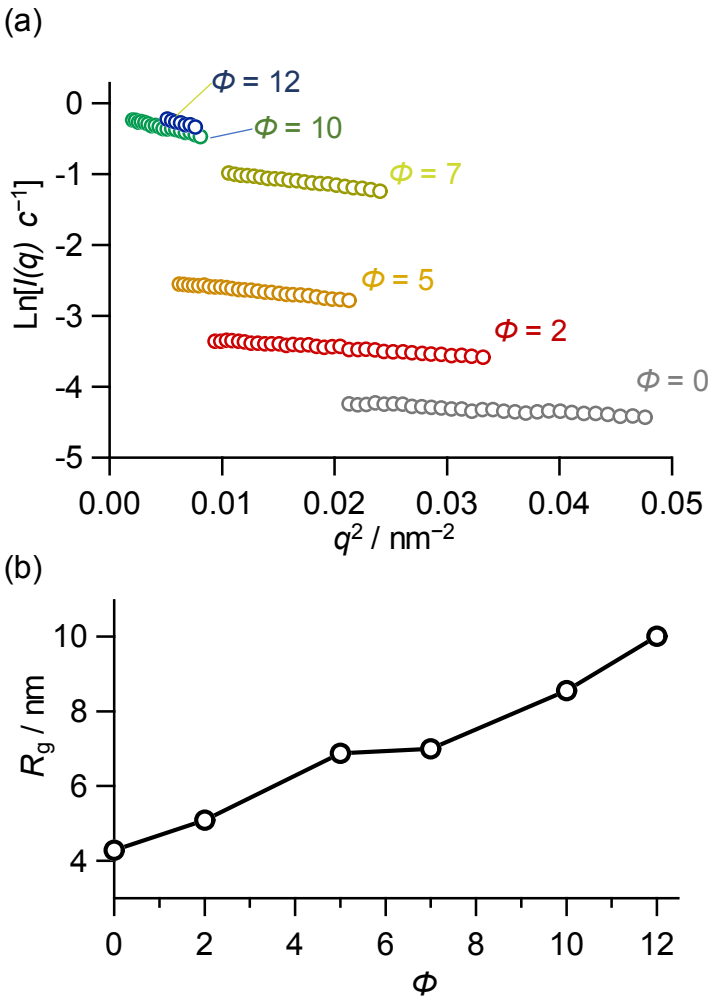


Figure S3. (a) Guinier plots for $PEG_{2k}C_{12}MA$ micelles (φ = 0: gray) and f-NPs (φ = 2: red, φ = 5: orange, φ = 7: yellow, φ = 10: green, φ = 12: blue). (b) R_g plotted against φ {= [DVB]/[$PEG_{2k}C_{12}MA$]} determined by Guinier analysis.