



Effect of Hydrophobicity on the Self-Assembly Behavior of Urea Benzene Derivatives in **Aqueous Solution**

Yuna Okamoto¹, Kosuke Morishita¹, Yasufumi Fuchi², Shigeki Kobayashi² and Satoru Karasawa ^{2,3,*} ^(D)

- 1 Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-Ku, Fukuoka 812-8582, Japan; yuna.o@abelia.ocn.ne.jp (Y.O.); k.morishita.0917@gmail.com (K.M.)
- 2 Faculty of Pharmaceutical Sciences, Showa Pharmaceutical University, Machida 194-8543, Japan; fuchi@ac.shoyaku.ac.jp (Y.F.); kobayasi@ac.shoyaku.ac.jp (S.K.)
- 3 PRESTO, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
- * Correspondence: karasawa@ac.shoyaku.ac.jp; Tel.: +81-42-721-1553

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Abstract: Urea benzene derivatives (UBD) with amphiphilic side chains showed self-assembly behavior in aqueous solution to form nanoparticles ~100 nm in size. Subsequent thermal treatment led to additional self-assembly of the nanoparticles due to dehydration of the amphiphilic side chains, producing microparticles. This self-assembly process was accompanied by a lower critical solution temperature (LCST) behavior, as revealed by the abrupt decrease in solution transmittance. In this study, three UBD (UBD-1–3) with different lengths of the alkyl segment in the amphiphilic side chain (namely, hexyl, heptyl, and octyl, respectively) were prepared to investigate the self-assembly behavior in aqueous solution. UBD-1–3 formed identical nanoparticles, with sizes in the 10~80 nm range but with different LCST values in the order 3 < 2 < 1. These results suggest a relationship between the hydrophobicity and the self-assembly behavior of UBD.

Keywords: nanoparticles; LCST; alkyl urea benzene framework; self-assembly

1. Introduction

Biomimetic chemistry can help in the further development of the medical field [1]. Artificial soft materials such as gels and nanoparticles are essential and have been applied in medical and pharmaceutical chemistry. For example, hydrogels [2,3] have been used in artificial joints, corneas, and stents. In addition, functional nanoparticles that can accumulate at a specific target site and release therapeutic agents have become essential tools in drug delivery systems (DDS) [4–6]. Because of their so-called "enhanced permeability and retention (EPR)" effect, that is, the ability to reach the interstitial spaces (10–500 nm) of tumor tissues [7–9], over the past three decades, nanoparticles have emerged as attractive DDS for anticancer drugs. Moreover, the acidic microenvironment of tumor cells, due to the Warburg effect [10,11], facilitates the accumulation of nanoparticles in the target tissue. We have previously reported the self-assembly behavior of urea benzene derivatives (UBD) in aqueous solution to form nanoparticles [12], which can undergo an additional heat-induced self-assembly to produce microparticles. This is accompanied by a lower critical solution temperature (LCST) behavior, resulting in a turbid solution. In addition, UBD have been used in our laboratory for the development of functional materials; namely, radical nanoparticles have been employed for metal-free magnetic resonance imaging contrast agents [13], and push-pull-type, aminoquinoline-based fluorescent probes have been constructed for bioimaging applications [14–18]. Among the latter, we have successfully developed a tumor-imaging probe activated by heat treatment [19].



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In this study, three **UBD** (**UBD-1–3**) with different alkyl chain lengths (hexyl, heptyl, and octyl, respectively) were prepared to investigate the self-assembly behavior in aqueous solution [20,21]. Although nanoparticle formation was observed in all cases, the existence of a critical aggregation concentration (CAC) was indicative of the effect of the alkyl chain length. Herein, we describe the relationship between the self-assembly behavior and the alkyl chain length of **UBD-1–3**.

2. Results and Discussion

2.1. Synthesis of UBD-1-3

UBD-1–3 contain an amphiphilic chain consisting of hexaethylene glycol and an alkyl chain of different lengths. For the synthesis of the amphiphilic chain, the corresponding dibromoalkane was reacted with hexaethylene glycol monomethyl ether under basic conditions. The resulting chain having a terminal bromine was converted to the phthalimide derivative, which was then reduced to obtain the corresponding amine. This was treated with phenyl isocyanate in toluene to afford the desired **UBD**, in which the phenyl group and the amphiphilic chain are linked by a urea moiety. The synthetic route to **UBD-1–3** is shown in Scheme 1.



(a) dibromoalkane, NaH, THF; (b) potassium pththalimide, DMF; (c) hydrazine monohydrate, EtOH; (d) phenyl isocyanate

Scheme 1. Synthetic route to UBD-1-3.

2.2. Self-Assembly Behavior in Aqueous Solution

2.2.1. ¹H NMR spectra in D_2O

The prepared **UBD-1–3** underwent self-assembly in aqueous solution to form aggregates. To determine the critical aggregation concentrations (CACs), the concentration dependence of the ¹H NMR spectra in D_2O was investigated, and the spectral changes for **UBD-1** having a hexyl chain are shown in Figure 1a,b.

The signals corresponding to the alkyl, ethylene glycol, and phenyl moieties were observed in the ranges of 1.1–1.6, 3.0–3.7, and 6.9–7.5 ppm, respectively. For concentrations between 0.25 and 20 mM, no chemical shift changes were observed, indicating that **UBD-1** was in the monomeric form in this concentration range. Above 20 mM, the proton peaks of the alkyl and ethylene glycol chains as well as some phenyl signals shifted to higher shielding and broadened, suggesting a concentration dependence. In contrast, the aromatic protons in the ortho position (H₀ in Scheme 1) were deshielded. These shielding [13,19,22] and deshielding shifts [13,19,23] typically derive from the formation of aggregates and the interaction via hydrogen bond between urea moiety and H₀ atoms in the phenyl ring, respectively. The chemical shift of the aromatic protons in the meta position (H_m in Scheme 1) was plotted as a function of the concentration, and a breaking point was observed at 16 mM (Figure 1c), which corresponds to the CAC of **UBD-1**. Notably, compared to **UBD-1**, **UBD-2** and **-3** with heptyl and octyl chains, respectively (Figure S1), showed a similar concentration dependence but different CAC values (9.0 and 2.0 mM, respectively), indicating that the CAC depended on the hydrophobicity of the amphiphilic chain. As expected, a longer chain was associated with a smaller CAC value. For all **UBD**, the ¹H NMR spectral changes with concentration are shown in Figure 1 and Figure S1, and the chemical shift changes of the meta protons are shown in Figure 1c. Moreover, the CAC and LCST values are summarized in Table 1.



Figure 1. ¹H NMR spectral changes with concentration of **UBD-1**. (a) ¹H NMR spectra in the chemical shift region of 0.0–7.8 ppm; and (b) enlarged aromatic region; (c) Plots of the H_m chemical shift as a function of the concentration for **UBD-1–3**. H_m indicates the aromatic protons in the meta position (Scheme 1).

2.2.2. Size Distribution and Morphology of **UBD-1–3** by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM)

The size distribution and morphology of the resulting **UBD-1–3** aggregates in aqueous solution were determined by DLS and TEM. The DLS spectrum of a 20 mM **UBD-1** solution at 20 °C showed a broad peak corresponding to a hydrodynamic diameter ($D_{\rm H}$) of approximately 30 nm (Figure 2a). At 90 °C, the peak shifted to about 200 nm, indicating that the aggregate size ($D_{\rm H}$ value) was temperature dependent, increasing with increasing temperature. The temperature dependence of the $D_{\rm H}$ values for **UBD-1** is illustrated in Figure S2, which also shows the transmittance changes (vide infra). A similar temperature dependence of the size distribution was observed for **UBD-2** and **-3** aggregates (10 mM). At 20 °C, **UBD-2** and **-3** had a size of approximately 20 nm and 70 nm, respectively, and at 30 °C, the $D_{\rm H}$ values shifted to larger sizes, namely, ~1500 and ~4000 nm, respectively (Figure 2b).

The temperature-dependent change in size distribution for **UBD-1** is shown in Figure 2a, and for all **UBD**, the temperature dependence of $D_{\rm H}$ is shown in Figure 2c.



Figure 2. (a) Size distribution of 20 mM **UBD-1** solution at 20 (blue line) and 90 °C (red line); (b) Size distribution of 10 mM **UBD-1** (red lines), 10 mM **UBD-2** (blue lines), and 10 mM **UBD-3** (green lines) solutions, below (solid lines) and above (dotted lines) the cloud point.

The TEM images of **UBD** were obtained using uranyl acetate as a negative staining agent and the dried samples prepared according to previous reports [12,13,19]. As can be seen from the images shown in Figure 3, for all **UBD**, spherical nanoparticles with sizes in the 10–80 nm range were observed. The size difference between DLS and TEM measurements could be attributed to the presence or absence of water molecules in the nanoparticles, indicating that **UBD-1–3** formed spherical nanoparticles 10–80 nm in size in aqueous solution. The TEM images of all **UBD** are shown in Figure 3.



Figure 3. TEM images of **UBD-1** (**a**,**b**), **-2** (**c**,**d**), and **-3** (**e**,**f**). The right panels (**b**,**d**,**f**) show enlarged images of the red dotted squares in left panels.

2.2.3. Additional Heat-Induced Self-Assembly

The hexaethylene glycol chain of **UBD** formed hydrogen bonds with water molecules and can undergo dehydration by thermal treatment, resulting in an entropy-driven self-assembly process [24,25]. To examine this heat-induced self-assembly behavior of the nanoparticles, the temperature dependence

of the transmittance was measured. For 10, 15, and 20 mM solutions of **UBD-1**, an abrupt decrease in transmittance was detected, with LCST at 71, 51, and 38 °C, respectively. On the other hand, at concentrations below 10 mM, the transmittance decreased gradually (Figure 4a). It should be noted that similar LCST behavior and concentration dependence were observed for **UBD-2** and **-3**. Namely, in the case of 5, 10, and 20 mM solutions of **UBD-2**, the LCSTs were found at 57, 29, and 26 °C, whereas for 3, 7, and 10 mM solutions of **UBD-3**, LCSTs at 27, 23, and 23 °C were observed. At a specific concentration (10 mM) of **UBD-1–3**, the LCSTs followed the order **UBD-3** < **2** < **1**, indicating that longer alkyl chains provide lower cloud points [26,27] due to hydrophobic effects (Figure 4b). The LCST behavior of **UBD** is shown in Figure 4 and Figure S3, and the LCST values are summarized in Table 1.



Figure 4. LCST behavior of (a) UBD-1 at given concentrations and (b) 10 mM solutions of UBD-1-3.

	UBD-1	UBD-2	UBD-3
		CAC	
	16 mM	9 mM	2 mM
Concentration/mM		LCST	
20	38 °C	26 °C	-
15	51 °C	-	23 °C
10	71 °C	29 °C	-
7	>80 °C	-	23 °C
5	>80 °C	57 °C	-
3	-	>80 °C	27 °C
1	>80 °C	-	-

Table 1. CAC Values and Concentration Dependence of the LCST Values of UBD.

3. Materials and Methods

3.1. Experimental

Instruments: Infrared spectra for freeze-dry samples of **UBD-1**, *-***2**, and *-***3** were recorded on a JASCO 420 FT-IR spectrometer (Figure S4) [28,29]. UV-Vis spectra were recorded on a JASCO V570 spectrometer equipped with a JASCO 420 temperature controller. ¹H and ¹³C NMR spectra were measured on a Bruker Biospin AVANCE III 500 spectrometer or a Varian 500 Fourier transform NMR spectrometer using CDCl₃, CD₃OD, or D₂O as a solvent and referenced to tetramethylsilane or 4,4-dimethyl-4-silapentane-1-sulfonic acid. DLS measurements were performed with a Malvern Zetasizer Nano ZS instrument with a helium-neon laser (wavelength: 633 nm) at a scattering angle of 173°. High-resolution electrospray ionization mass spectra were recorded using a Bruker Daltonics microTOF mass spectrometer. TEM images were obtained using a FEI Tecnai F20 transmission electron microscope operating at 200 kV. Samples for TEM observations were prepared by dropping the **UBD** solutions on a carbon-coated copper grid, followed by staining with uranyl acetate, and drying at room temperature. The LCST behavior of the **UBD** solutions was investigated by monitoring the change in the cloud point, which was determined by the inflection point of the transmittance at 800 nm as a function of temperature. The temperature of the sample in a cuvette was set at 10 $^{\circ}$ C and increased by increments of 1 $^{\circ}$ C up to a final temperature of 85 $^{\circ}$ C. Each measurement was obtained after maintaining the temperature for 120 s. Elemental analyses were performed using a Perkin Elmer 2400 II analyzer at the Center for Industry, University and Government Cooperation, Nagasaki University.

3.2. Materials

26-bromo-2,5,8,11,14,17,20-heptaoxahexacosane (Eg₆C₆Br)

To a solution of 1,6-dibromohexane (5.93 mL, 39.1 mmol) and 60% sodium hydride (0.939 g, 23.5 mmol) in 8.5 mL THF was added dropwise to a solution of hexaethylene glycol monomethyl ether (2.32 g, 7.83 mmol) in 8.5 mL THF while stirring in an ice bath, and the mixture was stirred overnight at room temperature. The resulting solution was diluted with a mixture of MeOH and saturated ammonium chloride aqueous solution and then extracted three times with Et₂O. The combined organic phases were dried over MgSO₄ and evaporated. The resulting residue was chromatographed on silica gel with n-hexane/AcOEt (100:5–100:30) as the eluent to afford **Eg₆C₆Br** as a colorless oil in 81.9% yield (2.95 g, 6.42 mmol). ¹H NMR (CDCl₃, 270 MHz) δ 3.63–3.26 (*m*, 24H), 3.59–3.53 (*m*, 2H), 3.46 (*t*, *J* = 6.8 Hz, 2H), 3.38 (*s*, 3H), 1.89–1.81 (*m*, 2H), 1.62–1.57 (*m*, 2H), 1.40–1.36 (*m*, 4H). The ¹H NMR spectrum was consistent with that previously reported [19].

27-bromo-2,5,8,11,14,17,20-heptaoxaheptacosane (Eg₆C₇Br)

Eg₆C₇Br was prepared in a manner similar to that described for **Eg₆C₆Br**, using dibromoheptane instead of dibromohexane. The reaction yield was 66.4%. ¹H NMR (CDCl₃, 500 MHz) δ 3.53–3.49 (*m*, 22H), 3.47–3.46 (*m*, 2H), 3.44–3.42 (*m*, 2H), 3.38 (*t*, *J* = 2.5 Hz, 2H), 3.23 (*s*, 3H), 1.49–1.46 (*m*, 2H), 1.37–1.36 (*m*, 2H), 1.30–1.27 (*m*, 6H). HRMS (ESI) Calcd. for C₂₀H₄₁BrNaO₇ [M + Na]⁺: *m/z* 495.1833, Found: 475.1819.

28-bromo-2,5,8,11,14,17,20-heptaoxaoctacosane (Eg₆C₈Br)

Eg₆C₈Br was prepared in a manner similar to that described for **Eg₆C₆Br**, using dibromooctane instead of dibromohexane. The reaction yield was 67.7%. ¹H NMR (CDCl₃, 500 MHz) δ 3.65–3.64 (*m*, 20H), 3.58–3.54 (*m*, 4H), 3.46–3.39 (*m*, 4H), 3.38 (*s*, 3H), 1.88–1.82 (*m*, 2H), 1.60–1.54 (*m*, 2H), 1.46–1.38 (*s*, 2H), 1.31 (*m*, 6H). HRMS (ESI) Calcd. for C₂₁H₄₃BrNaO₇ [M + Na]⁺: *m/z* 509.2090, Found: 509.2046.

2-(2,5,8,11,14,17,20-heptaoxahexacosan-26-yl)isoindoline-1,3-dione (Eg₆C₆NPht)

A mixture of Eg_6C_6Br (2.94 g, 6.40 mmol) and potassium phthalimide (1.30 g, 7.04 mmol) in 49.3 mL DMF was stirred at 110 °C for 4 h. After cooling to room temperature, the resulting solution was evaporated and diluted with water. The obtained mixture was extracted three times with Et₂O and the combined organic phases were dried over MgSO₄ and evaporated to afford Eg₆C₆NPht as a colorless oil in 73.5% yield (2.47 g, 4.70 mmol). IR (Neat on NaCl) 3465, 2932, 2862, 1716, 1606, 1459, 1399, 1360, 1302, 1251, 1104, 721 cm⁻¹. ¹H NMR (CDCl₃, 270 MHz) δ 7.86–7.83 (*m*, 2H), 7.72–7.69 (*m*, 2H), 3.70–3.61 (*m*, 20H), 3.58–3.53 (*m*, 4H), 3.46–3.41 (*t*, *J* = 6.75 Hz, 2H), 3.38 (*s*, 3H), 1.70–1.65 (*m*, 4H), 1.59 (*m*, 2H), 1.38–1.36 (*m*, 4H). The ¹H NMR spectrum was consistent with that previously reported [19].

2-(2,5,8,11,14,17,20-heptaoxaheptacosan-27-yl)isoindoline-1,3-dione (Eg₆C₇NPht)

Eg₆C₇NPht was prepared in a manner similar to that described for **Eg₆C₆NPht**, using **Eg₆C₇Br** instead of **Eg₆C₆Br**. The reaction yield was 76.7%. IR (neat on NaCl) 3517, 2930, 2863, 1771, 1713, 1635, 1467, 1438, 1396, 1369, 1108, 722 cm⁻¹. ¹H NMR (CDCl₃, 500 MHz) δ 7.85–7.83 (*dd*, *J* = 5.45, 3.05 Hz, 2H), 7.71–7.70 (*dd*, *J* = 5.45, 3.00 Hz, 2H), 3.69–3.62 (*m*, 20H), 3.57–3.54 (*m*, 4H), 3.44–3.42

(t, J = 6.78 Hz, 2H), 3.38 (s, 3H), 1.70-1.64 (m, 4H), 1.59-1.54 (m, 2H), 1.34-1.30 (m, 6H). HRMS (ESI) Calcd. for C₂₈H₄₅NNaO₉ [M + Na]⁺: m/z 562.2992, Found: 562.3199.

2-(2,5,8,11,14,17,20-heptaoxaoctacosan-28-yl)isoindoline-1,3-dione (Eg₆C₈NPht)

Eg₆C₈NPht was prepared in a manner similar to that described for **Eg₆C₆NPht**, using **Eg₆C₈Br** instead of **Eg₆C₆Br**. The reaction yield was 94.7%. ¹H NMR (CDCl₃, 500 MHz) δ 7.85–7.83 (*dd*, *J* = 5.45, 3.05 Hz, 2H), 7.71–7.70 (*dd*, *J* = 5.4, 3.00 Hz, 2H), 3.68–3.62 (*m*, 20H), 3.58–3.54 (*m*, 4H), 3.44–3.42 (*t*, *J* = 6.85 Hz, 2H), 3.38 (*s*, 3H), 1.68–1.64 (*m*, 2H), 1.58–1.53 (*m*, 2H), 1.33–1.30 (*m*, 10H). HRMS (ESI) Calcd. for C₂₉H₄₇NNaO₉ [M + Na]⁺: *m*/*z* 576.3149, Found: 576.3349.

2,5,8,11,14,17,20-heptaoxahexacosan-26-amine (Eg $_6C_6NH_2$)

To a solution of Eg_6C_6NPht (4.69 g, 8.92 mmol) in 40 mL EtOH, hydrazine monohydrate (1.73 mL, 35.7 mmol) was added dropwise, and the mixture was refluxed for 2 h. The resulting solution was cooled to room temperature and evaporated to afford a white solid. The residue was dissolved with Et₂O and filtered over Celite. After evaporation of the filtrate, the residue was chromatographed on silica gel with CH₂Cl₂/MeOH/trimethylamine (1:0.02:0.05) as the eluent to afford $Eg_6C_6NH_2$ as a pale yellow transparent oil in 97.8% yield (3.57 g, 9.03 mmol). IR (neat on NaCl) 3429, 2933, 2862, 1646, 1567, 1461, 1352, 1246, 1105, 938, 847 cm⁻¹. ¹H NMR (CDCl₃, 270 MHz) δ 3.66–3.62 (*m*, 20H), 3.59–3.53 (*m*, 4H), 3.45 (*t*, *J* = 8.1 Hz, 2H), 3.38 (*s*, 3H), 2.71 (*t*, *J* = 6.75 Hz, 2H), 1.74 (*m*, 2H), 1.59–1.56 (*t*, *J* = 4.05 Hz, 2H), 1.45–1.25 (*m*, 6H). HRMS (ESI) Calcd. for C₁₉H₄₂NO₇ [M + H]⁺: *m*/z 396.2961, Found: 396.3093.

2,5,8,11,14,17,20-heptaoxaheptacosan-27-amine (Eg₆C₇NH₂)

Eg₆C₇NH₂ was prepared in a manner similar to that described for **Eg₆C₆NH₂**, using **Eg₆C₇NPht** instead of **Eg₆C₆NPht**. The reaction yield was 86.2%. ¹H NMR (DMSO-*d*₆, 270 MHz) δ 3.53–3.50 (*m*, 24H), 3.49–3.30 (*m*, 2H), 3.37 (*s*, 3H), 2.71–2.69 (*t*, *J* = 5.0 Hz, 2H), 1.74 (*m*, 4H), 1.50–1.45 (*t*, *J* = 12.5 Hz, 2H), 1.40–1.30 (*m*, 4H), 1.19–1.13 (*m*, 2H). HRMS (ESI) Calcd. for C₂₀H₄₄NO₇ [M + H]⁺: *m/z* 410.3118, Found: 410.2994.

2,5,8,11,14,17,20-heptaoxaoctacosan-28-amine (Eg₆C₈NH₂)

Eg₆C₈NH₂ was prepared in a manner similar to that described for **Eg₆C₆NH₂**, using **Eg₆C₈NPht** instead of **Eg₆C₆NPht**. The reaction yield was 81.8%. ¹H NMR (DMSO-*d*₆, 500 MHz) δ 3.51–3.50 (*m*, 16H), 3.47–3.45 (*m*, 2H), 3.45–3.41 (*m*, 2H), 3.40–3.23 (*m*, 6H), 3.24 (*s*, 3H), 2.60–2.56 (*t*, *J* = 10 Hz, 2H), 1.49–1.46 (*m*, 2H), 1.40–1.36 (*m*, 2H), 1.30–1.20 (*m*, 6H). HRMS (ESI) Calcd. for C₂₁H₄₆NO₇ [M + H]⁺: m/z 424.3274, Found: 424.3145.

1-(2,5,8,11,14,17,20-heptaoxahexacosan-26-yl)-3-phenylurea (UBD-1)

To a solution of phenyl isocyanate (52.9 mg, 0.44 mmol) in 1 mL toluene, a solution of $Eg_6C_6NH_2$ (200 mg, 0.49 mmol) in 1 mL CH₂Cl₂ was added dropwise while stirring in an ice bath. The mixture was stirred overnight, allowing it to reach room temperature. The resulting solution was evaporated and chromatographed on silica gel with CH₂Cl₂/MeOH (10:1) as the eluent to afford 189 mg of **mono-PhC6** as a colorless oil in 82.6% yield. IR (neat on NaCl) 3348, 2931, 2864, 1696, 1668, 1597, 1553, 1500, 1441, 1350, 1311, 1238, 1109, 949, 850 cm⁻¹. ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.35 (*s*, 1H), 7.38–7.37 (*dd*, *J* = 8.7, 1.2 Hz, 2H), 7.21–7.18 (*t*, *J* = 8.0 Hz, 2H), 6.89–6.85 (*t*, *J* = 8.5 Hz, 1H), 6.09–6.07 (*t*, *J* = 5.6 Hz, 1H), 3.50–3.49 (*m*, 20H), 3.46–3.45 (*m*, 2H), 3.43–3.41 (*m*, 2H), 3.39–3.36 (*t*, *J* = 6.6 Hz, 2H), 3.23 (*s*, 3H), 3.08–3.03 (*q*, *J* = 12.7, 6.9 Hz, 2H), 1.51–1.48 (*m*, 2H), 1.43–1.40 (*m*, 2H), 1.30–1.29 (*m*, 4H). ¹³C NMR (DMSO-*d*₆, 126 MHz) δ 155.21, 140.61, 128.62, 120.90, 117.56, 71.29, 70.29, 69.81, 69.60, 69.50, 58.06, 39.52, 29.76, 29.21, 26.25, 25.45. HRMS (ESI) Calcd. for C₂₆H₄₆N₂NaO₈ [M + Na]⁺: *m*/z 537.3152, Found: 537.3259.

1-(2,5,8,11,14,17,20-heptaoxaheptacosan-27-yl)-3-phenylurea (UBD-2)

UBD-2 was prepared in a manner similar to that described for **mono-PhC6**, using **Eg₆C₇NH₂** instead of **Eg₆C₆NH₂**. The reaction yield was 91.5%. IR (neat on NaCl) 3306, 2929, 2864, 1686, 1597, 1551, 1500, 1442, 1351, 1313, 1239, 1106, 952, 849 cm⁻¹. ¹H NMR (DMSO-*d*₆, 500 MHz) δ 8.36 (*s*, 1H), 7.37–7.35 (*dd*, *J* = 8.7, 1.2 Hz, 2H), 7.20–7.18 (*t*, *J* = 4.3 Hz, 2H), 6.87–6.86 (*t*, *J* = 1.0 Hz, 1H), 6.10–6.08 (*t*, *J* = 5.6 Hz, 1H), 3.50–3.49 (*m*, 24H), 3.47–3.45 (*m*, 2H), 3.43–3.41 (*m*, 2H), 3.38–3.34 (*t*, *J* = 10.3 Hz, 2H), 3.23 (*s*, 3H), 3.08–3.04 (*q*, *J* = 12.7, 6.8 Hz, 2H), 1.50–1.47 (*m*, 2H), 1.43–1.40 (*m*, 2H), 1.28 (*m*, 6H). ¹³C NMR (DMSO, 126 MHz) δ 155.23, 140.60, 128.64, 120.93, 117.58, 71.29, 70.32, 69.81, 69.60, 69.50, 58.07, 39.25, 29.72, 29.17, 28.61, 26.37, 25.66. HRMS (ESI) Calcd. for C₂₇H₄₈N₂NaO₈ [M + Na]⁺: *m*/z 537.3308, Found: 551.3323. Anal. Calcd. for C₂₇H₄₈N₂O₈: C, 61.34; H, 9.15; N, 5.30, Found: C, 61.33; H, 9.26; N, 5.44.

1-(2,5,8,11,14,17,20-heptaoxaoctacosan-28-yl)-3-phenylurea (UBD-3)

UBD-3 was prepared in a manner similar to that described for **mono-PhC6**, using **Eg₆C₈NH**₂ instead of **Eg₆C₆NH**₂. The reaction yield was 45.6%. IR (neat on KBr) 3303, 2927, 2860, 1685, 1597, 1551, 1450, 1441, 1351, 1312, 1236, 1106, 951, 849 cm⁻¹. ¹H NMR (DMSO- d_6 , 500 MHz) δ 8.35 (*s*, 1H), 7.37–7.35 (*dd*, *J* = 8.6, 1.0 Hz, 2H), 7.21–7.18 (*t*, *J* = 7.9 Hz, 2H), 6.88–6.85 (*t*, *J* = 7.9 Hz, 1H), 6.09–6.07 (*t*, *J* = 5.6 Hz, 1H), 3.50–3.46 (*m*, 24H), 3.46–3.44 (*m*, 2H), 3.43–3.41 (*m*, 2H), 3.38–3.36 (*t*, *J* = 6.6 Hz, 2H), 3.23 (*s*, 3H), 3.08–3.04 (*q*, *J* = 12.8, 6.8 Hz, 2H), 1.50–1.46 (*m*, 2H), 1.42–1.40 (*m*, 2H), 1.27 (*m*, 8H). ¹³C NMR (DMSO- d_6 , 126 MHz) δ 155.22, 140.60, 128.63, 120.92, 117.57, 71.30, 70.33, 69.81, 69.60, 69.50, 58.07, 39.25, 29.76, 29.22, 28.88, 28.79, 26.36, 25.64. HRMS (ESI) Calcd. for C₂₇H₄₈N₂NaO₈ [M + Na]⁺: *m*/z 565.3465, Found: 565.3577. Anal. Calcd. for C₂₈H₅₀N₂O₈: C, 61.97; H, 9.29; N, 5.16, Found: C, 61.68; H, 9.43; N, 5.04.

4. Conclusions

To investigate the self-assembly behavior of **UBD** containing an amphiphilic chain in aqueous solution, three **UBD** (**UBD-1–3**) with different alkyl chain lengths, namely, hexyl, heptyl, and octyl, respectively, were prepared. All **UBD** formed identical spherical nanoparticles at concentrations above the CAC. Moreover, the size of the nanoparticles increased with increasing temperature because of the enhanced hydrophobic interactions due to dehydration of the ethylene glycol chains. In addition, the transmittance of the **UBD** solutions abruptly decreased, indicating an LCST behavior. A comparison of the CAC and LCST of the three **UBD** revealed that **UBD-3** exhibited the smallest CAC and the largest LCST values, suggesting a relationship between the hydrophobicity of the **UBD** and its self-assembly behavior. Studies with varying concentrations of **UBD** (Figure 5, left scheme) revealed an equilibrium between the monomer and the nanoparticles. In the other hand, by varying the temperature (Figure 5, right scheme), the dehydration process was responsible for the formation of larger particles, which were in equilibrium with the nanoparticles. In the future, we plan to synthesize nanoparticles having functional groups for bioimaging [30] and investigate the self-assembly behavior of nanoparticles bearing different numbers of amphiphilic side chains.



Figure 5. Predicted mechanism of the self-assembly of UBD in aqueous solution.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/8/7/1080/s1, Concentration dependence of the ¹H NMR spectra of **UBD-2** and **-3**. Temperature dependence of the $D_{\rm H}$ values and transmittance changes for **UBD-1**. Temperature dependence of the LCST behavior of **UBD-2** and **-3**. FTIR spectra of the freeze-dry samples of **UBD-1**, **-2**, and **-3**.

Author Contributions: S.K. designed the experiments, conceived and supervised the project, analyzed the final data, and wrote the manuscript. Y.O., K.M., Y.F., and S.K. performed the experiments and analyzed the data to investigate the properties and mechanistic behavior of the materials. Y.F. checked the data and the manuscript, and discussed the results with the corresponding author.

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