# Preparation of Amphiphilic Diblock Copolymers with Pendant Hydrophilic Phosphorylcholine and Hydrophobic Dendron Groups and Their Self-Association Behavior in Water

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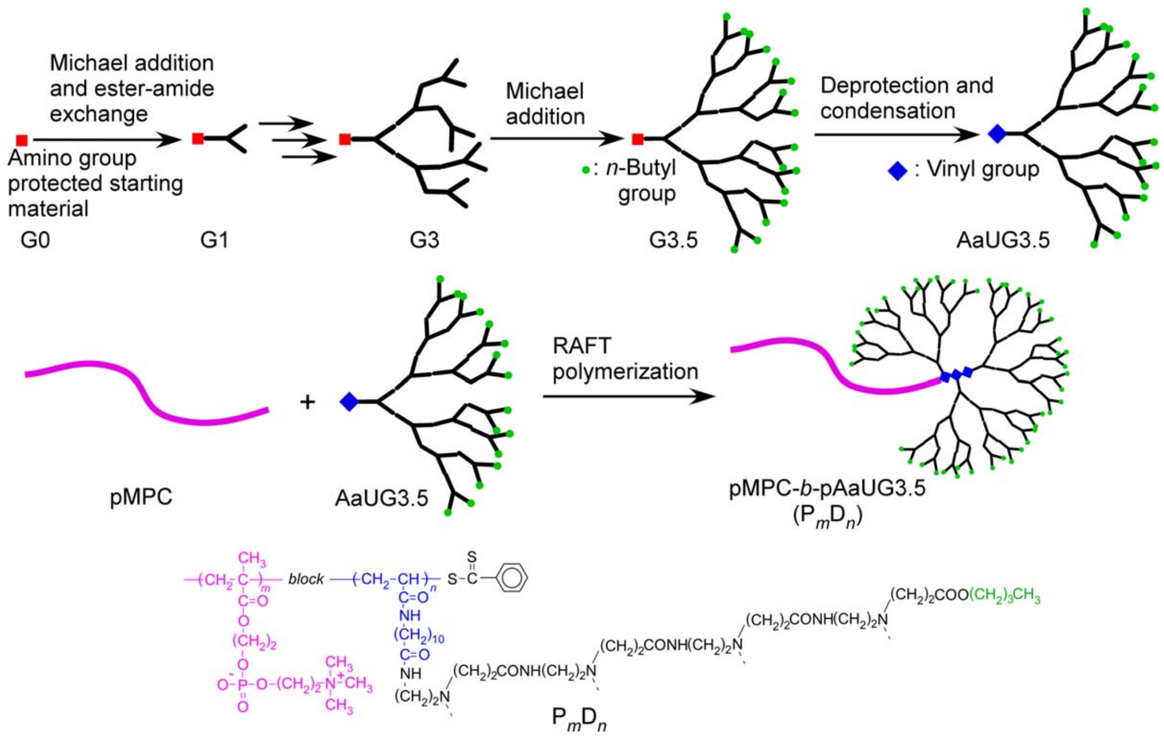
**ABSTRACT**: Generation 3.5 poly(amido amine) dendron (G3.5) with 16 *n*-butyl terminal groups containing an acrylamide monomer (AaUG3.5) was prepared by condensation between an amino focal group in G3.5 and 11-acrylamidoundecanoic acid. AaUG3.5 was polymerized using poly(2-methacryloyloxyethyl phosphorylcholine) (pMPC)-based macro-chain transfer agent via reversible addition-fragmentation chain transfer (RAFT) radical polymerization to obtain amphiphilic diblock copolymers with different compositions. The diblock copolymers ($P_mD_n$) were composed of a hydrophilic pMPC block and hydrophobic pendant dendron-bearing block, where P and D represent pMPC and pAaUG3.5, respectively, and *m* and *n* represent the degree of polymerization for each block, respectively. $P_{296}D_1$ and $P_{98}D_3$ formed vesicles and large compound micelles and vesicles, respectively, which was confirmed by light scattering measurements and transmission electron microscopic (TEM) observations. The large compound micelles formed from $P_{98}D_3$ could not incorporate hydrophilic guest polymer molecules, because the aggregates did not have a hydrophilic hollow core. In contrast, the vesicles formed from $P_{269}D_1$ could incorporate hydrophilic guest polymer molecules into the hollow core. © 2013 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. **2013**, *51*, 4923–4931

**KEYWORDS**: association; dendrimers; living radical polymerization; self-organization; water-soluble polymers

**INTRODUCTION** Amphiphilic block copolymers form different types of self-assemblies, such as dynamic core-shell spherical micelles, frozen crew-cut micelles, rods, or vesicles, because of hydrophobic interactions between the hydrophobic blocks in aqueous solution.[1–6] Amphiphilic diblock copolymers composed of hydrophobic poly(*n*-butyl acrylate) (PBA) and hydrophilic poly(acrylic acid) blocks form spherical polymer micelles composed of a PBA core in water.[7] Thermoresponsive diblock copolymers composed of poly(*N*,*N*-dimethylacrylamide) (PDMAEMA) and poly(*N*-isopropylacrylamide) (PNIPAM) exhibit specific morphological changes in the aggregates at temperatures above the lower critical solution temperature (LCST) in water depending on the hydrophilic PDMAEMA mass fraction.[8] As the PDMAEMA content decreased from 68, 48, and 36 wt % in the diblock copolymer, the diblock copolymer formed spherical core-shell micelles, a mixture of spherical and worm-like micelles, and vesicular structures, respectively. Self-assembly of amphiphilic block copolymers in water strongly depended on the molecular weight balance of hydrophilic/hydrophobic blocks. Furthermore, the chemical structure of each block in the amphiphilic block copolymer also affected the self-assemblies.

Dendrimers are of particular interest as polymers because they have a well-defined architecture, wide variety of functionality in a single macromolecule,[9,10] a large number of terminal groups, and an interior nanoporous nature in higher generations.[11] Furthermore, dendrimers are ideal building blocks for polymer architecture, because their structure can be controlled precisely. A combination of linear and dendritic blocks is an interesting approach for building

Additional Supporting Information may be found in the online version of this article.




**SCHEME 1** Synthesis of pMPC-*b*-pAaUG3.5 ($P_mD_n$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

amphiphilic block copolymers that form supramolecular aggregates in solution.[12–14] The synthesis and characterization of this type of dendrimeric diblock copolymer with one linear block and one dendritic block have been reported previously.[15–19]

van Hest et al.[20,21] reported the preparation of block copolymers with linear hydrophobic polystyrene and hydrophilic dendritic poly(propylene imine). The interesting aspect of the work was a morphology change in the aqueous phase from a vesicle to a rod and to a spherical micelle with increasing generation of the hydrophilic poly(propylene imine). Barrio et al.[22] synthesized amphiphilic linear-dendritic block copolymers (PEG*m*-AZO*n*,), composed of hydrophilic poly(ethylene glycol) (PEG) blocks with different molecular weights and hydrophobic azobenzene-containing dendrons based on 2,2-*bis*(hydroxymethyl)propionic acid, where *m* represents the degree of polymerization of PEG and *n* is the number of azobenzene units at the periphery of the dendron. The polymeric aggregates were formed by adding water to solutions of block copolymers in dioxane. Core-shell structured nanofibers were formed from the copolymer PEG45-AZO2. The coexistence of sheet-like aggregates and tubular micelles was detected in a solution of the copolymer PEG45-AZO8. The tubular micelles may be intermediates in the sheet-like-aggregate-to-vesicle transition. Polymer vesicles were observed from the copolymer PEG45-AZO16.

In most studies of diblock copolymers with one linear block and one dendritic block, the diblock copolymers were synthesized by polymerization of a linear tail from the focal point of the dendron. Head-to-tail polycation block copolymers are prepared in two steps, synthesis of the poly(amido amine) (PAMAM) dendron block and polymerization of the poly(L-lysine) block from the PAMAM dendron block.[23] Other examples include poly(2-methyl-2-oxazoline)-PAMAM dendrimer[24] and linear PEG-dendritic copolymers.[25]

PAMAM dendrimers are nanoscopic spherical macromolecules composed of polyamidoamino units with repeating dendritic branching. PAMAM dendrimer can incorporate guest molecules, such as platina,[26] gold,[27,28] palladium nanoparticles,[29] and organic compounds such as phenol blue,[30,31] into the interior formed by the branch chains. PAMAM dendrimers incorporating inorganic particles can play a role in recyclable catalysis. And PAMAM dendrons can interact with DNA, and so may be useful as carriers in biological delivery systems.[32–34]

A pendant hydrophobic dendron-containing monomer was synthesized and polymerized using a hydrophilic linear macro-chain transfer agent (macro-CTA) to prepare amphiphilic diblock copolymers composed of a linear polymer block and dendron-bearing block via reversible addition-fragmentation chain transfer (RAFT) radical polymerization (Scheme 1). This type of amphiphilic diblock copolymer forms different types of self-assemblies with high functionality from traditional linear amphiphilic diblock copolymers. In the present study, a generation 3.5 PAMAM dendron (G3.5) with 16 hydrophobic *n*-butyl-terminal-group-bearing acrylamide monomers (AaUG3.5) was prepared by condensation reaction between an amino group in the focal point of G3.5

and the carboxylic acid group in 11-acrylamidoundecanoic acid. Poly(2-methacryloyloxyethyl phosphorylcholine) (pMPC) was chosen as the linear hydrophilic block. Polymers containing MPC units exhibit excellent biocompatibility and antithrombogenicity. These types of polymers have been applied clinically in artificial hearts, artificial hip joints, and cardiovascular stents, because of their biocompatibility and safety profile.[35–37] Amphiphilic diblock copolymers (pMPC-*b*-pAaUG3.5 ($P_mD_n$)) composed of hydrophilic pMPC linear blocks and hydrophobic pAaUG3.5 blocks were prepared via RAFT polymerization, and their self-association behavior in water was investigated.

## EXPERIMENTAL

### Materials

Trifluoroacetic acid (TFA, 98%), 4,4′-azobis(4-cyanovaleric acid) (V-501, 98%), *N*-hydroxysuccinimide (HO-NSu, 98%), *N,N′*-diisopropylcarbodiimide (DIC, 99%), *N,N*-dimethylformamide (DMF, 99%), and dimethyl sulfoxide (DMSO, 99%) were purchased from Wako Pure Chemical Industries. DMF and DMSO were dried over 4 Å molecular sieves and distilled under reduced pressure. Methanol was dried over 4 Å molecular sieves and distilled. Then 11-acrylamidoundecanoic acid (11-AaU)[38] and 4-cyanopentanoic acid dithiobenzoate (CPD)[39] were synthesized according to previously reported procedures; 2-methacryloyloxyethyl phosphorylcholine (MPC) was synthesized as previously reported and recrystallized from acetonitrile.[40] *N*-phenyl-1-naphthylamine (PNA) (98%) was purchased from Tokyo Chemical Industry. Texas red-labeled dextran (Texas red-Dex, molecular weight (MW): 3000) was purchased from Life Technologies Corporation. Water was purified using a Millipore Milli-Q system. Other reagents were used as received.

### Removal of *tert*-Butoxy Carbonyl (Boc) Group from G3.5 (RG3.5)

*n*-Butyl terminal group containing Boc-PAMAM dendron (G3.5, 250 mg, 0.066 mmol) was dissolved in TFA (3.32 g, 29.2 mmol) and stirred for 3 h at room temperature. TFA was removed under vacuum, and chloroform (12 mL) and triethylamine (0.19 g, 1.88 mmol) were added to the residue. Distilled water (10 mL) was added to the solution, and the chloroform phase was collected after stirring. The chloroform was then removed under vacuum. The product obtained was purified through a Sephadex LH-20 column using methanol as eluent. PAMAM dendron (RG3.5) having a primary amino group at the focal point was obtained as a yellow oil (200 mg, 82.0%). IR ($CaF_2$, $\nu$, $cm^{-1}$) 1750 (C=O), 2800 ($CH_2$), 2980 ($CH_3$), 3380 (NH).

$^1$H NMR ($CDCl_3$, $\delta$, ppm) 0.95 (*t*, -$CH_2CH_3$), 1.45 (*m*, -$CH_2CH_2CH_3$), 1.63 (*m*, -$CH_2CH_2CH_3$), 2.42 (*m*, -$CH_2$CO-), 2.56 (*m*, -CONH$CH_2CH_2$- and $H_2NCH_2CH_2$-), 2.76 (*m*, -$CH_2CH_2$CO-), 3.31 (*m*, -CONH$CH_2$- and $H_2NCH_2$-), 4.08 (*m*, -O$CH_2$-). $^{13}$C NMR ($CDCl_3$, DEPT, $\delta$, ppm) 13.3 ($CH_3$), 19.8 ($CH_2$), 30.3 ($CH_2$), 32.5 ($CH_2$), 37.5 ($CH_2$), 49.8 ($CH_2$), 50.1 ($CH_2$), 52.5 ($CH_2$), 64.0 ($CH_2$), 172.8 (quaternary).

### Synthesis of Dendron-Containing Monomer (AaUG3.5)

HO-NSu (0.113 g, 0.978 mmol), DIC (0.130 g, 1.03 mmol), and 11-AaU (0.208 g, 0.814 mmol) were dissolved in DMF (7 mL), and the mixture was stirred for 4 h at 0 °C. Then, RG3.5 (0.200 g, 0.054 mmol) dissolved in DMSO (7 mL) and triethylamine (0.121 g, 1.20 mmol) was added to the solution, which was stirred for 5 days at room temperature. The product obtained was purified using a Sephadex LH-20 column with methanol as eluent. The monomer (AaUG3.5) was obtained as a yellow oil (180 mg, 83.0%). IR ($CaF_2$, $\nu$, $cm^{-1}$) 1755 (C=O), 2850 ($CH_2$), 2980 ($CH_3$), 3380 (NH).

$^1$H NMR (DMSO-$d_6$, $\delta$, ppm) 0.95 (*m*, —$CH_2CH_3$), 1.33 (*m*, —$CH_2(CH_2)_6CH_2$—), 1.45 (*m*, —$CH_2CH_2CH_3$), 1.51 (*m*, —$CH_2(CH_2)_8$CONH—), 1.54 (*m*, —$(CH_2)_9CH_2CH_2$CONH—), 1.63 (*m*, —$CH_2CH_2CH_3$), 2.13 (*t*, —$(CH_2)_9CH_2$CONH—), 2.31 (*m*, —N$CH_2CH_2$CONH—), 2.52 (*m*, —CONH$CH_2CH_2$N—), 2.74 (*m*, —N$CH_2CH_2$CO—), 3.19 (*m*, —$CH_2CH_2N(CH_2)_2$—), 3.48 (*m*, —CONH$CH_2(CH_2)_9$—) 4.08 (*t*, —COO$CH_2$—), 5.62 (m, $CH_2$=CH—), 6.15 (*m*, $CH_2$=CH—), 6.38 (*m*, $CH_2$=C*H*—).

$^{13}$C NMR (DMSO-$d_6$, DEPT, $\delta$, ppm) 13.3 ($CH_3$), 19.5 ($CH_2$), 26.3 ($CH_2$), 27.1 ($CH_2$), 29.5 ($CH_2$), 31.0 ($CH_2$), 32.8 ($CH_2$), 36.8 ($CH_2$), 37.5 ($CH_2$), 39.9 ($CH_2$), 49.9 ($CH_2$), 51.2 ($CH_2$), 52.8 ($CH_2$), 64.5 ($CH_2$), 125.1 (CH), 131.1 ($CH_2$), 166.9 (quaternary), 173.0 (quaternary), 177.5 (quaternary).

### Synthesis of $pMPC_{98}$ Macrochain Transfer Agent ($pMPC_{98}$ Macro-CTA)

A typical procedure for preparation of pMPC macro-CTA follows. MPC (10.0 g, 0.03 mol) was dissolved in water (58.3 mL), and the solution added to methanol (6.00 mL) containing CPD (0.093 g, 0.34 mmol) and V-501 (0.037 g, 0.13 mmol). The mixture was purged with argon gas for 30 min, and then heated at 70 °C for 2 h. After the polymerization, a portion of the solution was removed for $^1$H NMR to determine the conversion rate (98.6%). The solution was dialyzed (Spectra Pore; MWCO 1000 Da) against pure water for 2 days. After freeze-drying, the polymer ($pMPC_{98}$) was recovered as a pink powder (9.53 g, 95.3%). The number-average molecular weight ($M_n$(NMR)) and number-average degree of polymerization (DP) of $pMPC_{98}$ were $2.97 \times 10^4$ and 98, respectively, as estimated from $^1$H NMR. The number-average molecular weight ($M_n$(GPC)) and molecular weight distribution ($M_w/M_n$) estimated from gel-permeation chromatography (GPC) were $1.91 \times 10^4$ and 1.05, respectively.

$^1$H NMR ($D_2O$, $\delta$, ppm) 0.8–1.2 (*m*, main chain —C$CH_3$), 1.5–2.3 (*m*, main chain —$CH_2$—), 3.2–3.4 (*s*, —$N(CH_3)_3$), 3.7 (*m*, —$CH_2N(CH_3)_3$), 4.1 (*m*, —O$CH_2CH_2$O—), 4.2 (*m*, —O$CH_2$ $CH_2$O—), 4.3 (*m*, —O$CH_2CH_2N(CH_3)_3$), 7.5–8.1 (*m*, terminal phenyl protons). $pMPC_{296}$ was prepared and purified in a manner similar to that of the polymer described above.

### Synthesis of Amphiphilic Diblock Copolymer ($pMPC_{98}$-b-PAaUG3.5)

A typical procedure for block copolymerization follows. $pMPC_{98}$ macro-CTA (180 mg, 0.006 mmol, $M_n$(NMR) = 2.97 $\times 10^4$), AaUG3.5 (118 mg, 0.030 mmol), and AIBN (0.197

mg, 0.001 mmol) were dissolved in methanol (10.0 mL). The solution was purged with argon gas for 30 min, and then heated at 60 °C for 24 h. The conversion was 58.1%, as estimated by $^1$H NMR. The product obtained was purified using a Sephadex LH-20 column with methanol as eluent. The product obtained was dissolved in water, and the solution was dialyzed (Spectra Pore; MWCO 1000 Da) against pure water for one day. After freeze-drying, the polymer (pMPC$_{98}$-*b*-pAaUG3.5$_3$) was recovered as a pink powder (200 mg, 67.1%). DP of the AaUG3.5 block was 3, which was estimated from $^1$H NMR.

$^1$H NMR (methanol-$d_4$, $\delta$, ppm) 0.8-1.2 (*m*, -CH$_2$C*H*$_3$ and main chain -CC*H*$_3$), 1.31 (*m*, -CH$_2$(C*H*$_2$)$_6$CH$_2$-, and main chain -C*H*$_2$-), 1.40 (*m*, -CH$_2$C*H*$_2$CH$_3$), 1.52 (*m*, -C*H*$_2$(CH$_2$)$_6$C*H*$_2$-), 1.58 (*m*, -C*H*$_2$CH$_2$CH$_3$), 1.7-2.1 (*m*, main chain -C*H*$_2$-), 2.18 (*m*, -(CH$_2$)$_9$C*H*$_2$CONH-), 2.35 (*m*, -NCH$_2$C*H*$_2$CO-), 2.45 (*m*, -CH$_2$C*H*$_2$COO-), 2.58 (*m*, -C*H*$_2$N(C*H*$_2$)$_2$-), 2.80 (*m*, -NC*H*$_2$CH$_2$CO-), 3.30 (*m*, -NHC*H*$_2$CH$_2$- and -C*H*$_2$N(CH$_3$)$_3$), 3.55 (*m*, -CONHC*H*$_2$(CH$_2$)$_9$-), 3.70 (*m*, -C*H*$_2$N(CH$_3$)$_3$), 4.08(m, -OC*H*$_2$-CH$_2$O-), 4.18 (*m*, -OCH$_2$C*H*$_2$O-), 4.32 (*m*, -OC*H*$_2$CH$_2$N(CH$_3$)$_3$). pMPC$_{296}$-*b*-pAaUG3.5$_1$ was prepared and purified in a manner similar to that of the polymer described above.

### Sample Preparations

The diblock copolymer (0.5 mg) was dissolved in pure water (5 mL), and the solution was allowed to stand overnight at room temperature to achieve complete dissolution. The sample solution was filtered with a 0.8 μm pore size filter before measurements.

### Incorporation of Guest Molecules

The diblock copolymer (0.5 mg) was dissolved in methanol (5 mL), and the solution added to an aqueous solution (5 mL) of Texas red-Dex (0.1 mg). Methanol in the solution was evaporated under reduced pressure. The aqueous solution was dialyzed against pure water using a polycarbonate membrane with a 50 nm pore size (Harvard, polycarbonate membrane). Fluorescence spectra were measured for the aqueous solution inside the dialysis tube. The amount of Texas red-Dex was determined by fluorescence measurements based on a calibration curve with known concentrations of Texas red-Dex in water. Guest molecule-loaded content (LC) and guest molecule-loaded efficiency (LE) were calculated using the following equations.

$$\mathrm{LC} = (\text{weight of loaded guest} / \text{weight of polymer}) \times 100\% \tag{1}$$

$$\mathrm{LE} = (\text{weight of loaded guest} / \text{weight of guest in feed}) \times 100\% \tag{2}$$

## INSTRUMENTS

$^1$H and $^{13}$C NMR spectra were obtained with a Bruker DRX-500 spectrometer operating at 500.13 MHz. Sample solutions for $^1$H NMR were prepared in D$_2$O, CDCl$_3$, or methanol-$d_4$. Fourier transform-infrared (FT-IR) spectra were obtained using a Jasco FT/IR-4200 spectrophotometer on a CaF$_2$ optical crystal. GPC measurements for pMPC$_m$ were performed using a refractive index (RI) detector equipped with a Shodex 7.0 $\mu$m bead GF-7 M HQ column (exclusion limit $\sim 10^7$) working at 40 °C under a flow rate of 0.6 mL/min. A phosphate buffer (pH 9) containing 10 vol % acetonitrile was used as eluent. The values of $M_n$ and $M_w/M_n$ for pMPC$_m$ were calibrated using standard sodium polystyrene sulfonate samples of 11 different molecular weights ranging from 1.37 $\times$ 10$^3$ to 2.16 $\times$ 10$^6$. Light scattering measurements were obtained using an Otsuka Electronics Photal DLS-7000HL light scattering spectrometer equipped with a multi-$\tau$ digital time correlator (ALV-5000E). A He-Ne laser (10.0 mW at 632.8 nm) was used as a light source. Sample solutions for light scattering measurements were filtered by a 0.8 $\mu$m pore size membrane filter. To obtain the relaxation time distribution ($\tau A(\tau)$) in dynamic light scattering (DLS) measurements, inverse Laplace transform (ILT) analysis was performed using the REPES algorithm:[41]

$$g^{(1)}(t) = \int A(\tau) \exp(-t/\tau) \mathrm{d} \ln \tau \tag{3}$$

where $\tau$ is relaxation time and $g^{(1)}(t)$ is the normalized autocorrelation function. The relaxation rate ($\Gamma = \tau^{-1}$) is a function of polymer concentration ($C_p$) and scattering angle ($\theta$). The diffusion coefficient in the limit of zero angle ($D$) was calculated from $D = (\Gamma/q^2)_{q \to 0}$. The hydrodynamic radius ($R_h$) was obtained using the Stokes-Einstein equation, $R_h = k_B T / 6\pi\eta D_0$, where $k_B$ is the Boltzmann constant, $T$ is absolute temperature, and $\eta$ is solvent viscosity. The details of DLS instrumentation and theory have been described previously.[42] Static light scattering (SLS) measurements were obtained with an Otsuka Electronics DLS-7000 instrument equipped with a He-Ne laser (633 nm) in water at 25 °C The refractive index increment ($\mathrm{d}n/\mathrm{d}C_p$) in water at 25 °C for polymers was determined with an Otsuka Electronics DRM-1020 refractometer operating at 633 nm.[43] Transmission electron microscopic (TEM) photographs were taken on a Jeol JEM-2000 microscope operating at 200 kV. Samples for TEM were prepared by placing one drop of the aqueous solution on a copper grid coated with thin films of Formvar. Excess solution was blotted using filter paper. The samples were stained by sodium phosphotungstate and dried under vacuum for one day. Fluorescence spectra were obtained using a Hitachi F-2500 fluorescence spectrophotometer equipped with a magnetic stirrer using a 1.0 cm path length quartz cell. The maximum fluorescence wavelength of PNA in the copolymer solutions of various $C_p$ was plotted against $C_p$. Fluorescence spectra of PNA were obtained with excitation at 330 nm. Excitation and emission slit widths were maintained at 20 and 5 nm, respectively. Fluorescence spectra of Texas red-dex were measured with excitation at 550 nm. Excitation and emission slit widths were maintained at 10 and 20 nm, respectively.

## RESULTS AND DISCUSSION

### Preparation of Diblock Copolymers Containing Pendant Dendron Groups

Ethylenediamine with one amino group protected by a Boc group underwent Michael addition using a large excess of

**TABLE 1** Number-Average Molecular Weight ($M_n$), Number-Average Degree of Polymerization (DP), and Polydispersity Index ($M_w$/$M_n$) for the Polymers

| | $M_n$(theo) × $10^{-4}$ | $M_n$(NMR) × $10^{-4}$ | DP (NMR) | $M_n$(GPC) × $10^{-4}$ | $M_w/M_n$ |
|---|---|---|---|---|---|
| $pMPC_{98}$ | 2.97 | 2.97 | 100 | 1.91 | 1.05 |
| $pMPC_{296}$ | 8.73 | 8.73 | 296 | 2.94 | 1.05 |
| $P_{98}D_3$ | 3.94 | 3.94 | 3[a] | –[b] | –[b] |
| $P_{296}D_1$ | 9.12 | 9.12 | 1[a] | –[b] | –[b] |

[a] DP of pAaUG3.5 block.

[b] Values could not be obtained because the $P_mD_n$ did not molecularly dissolve in GPC eluent of phosphate buffer containing 10 vol % acetonitrile.

methyl acrylate to yield G0.5. The G0.5 obtained underwent an ester-amide exchange reaction using a large excess of ethylenediamine to produce G1.0. The third-generation PAMAM dendron (G3.0) was synthesized by repeating these reactions alternately. Hydrophobic PAMAM dendron (G3.5), which has 16 *n*-butyl terminal groups, was synthesized by Michael addition using G3.0 and excess *n*-butyl acrylate. The detailed synthesis method was described in Supporting Information. The Boc group of G3.5 was removed using TFA to obtain RG3.5. The carboxylic acid group in 11-AaU was reacted with the amino group in the focal point of RG3.5 by condensation reaction to yield pendant hydrophobic PAMAM dendron-containing acrylamide monomer (AaUG3.5). Products of the syntheses were confirmed with $^1$H and $^{13}$C NMR and IR spectra (Supporting Information Figs. S1–S9).

Preparation of $pMPC_{98}$ and $pMPC_{296}$ was confirmed by $^1$H NMR and GPC analyses. Supporting Information Figure S10 shows the $^1$H NMR spectra for $pMPC_{98}$ and $pMPC_{296}$ in $D_2O$. The resonance bands observed at 3.1 to 4.5 ppm were attributed to phosphorylcholine moieties, while those at 0.7 to 2.4 ppm were assigned to the methylene and α-methyl protons of the main chain. Resonance peaks observed at 7.5 to 8.1 ppm were assigned to the terminal phenyl protons. The DP values ($=m$ in $P_mD_n$) for the pendant methylene and terminal phenyl protons were calculated to be 98 and 296 from the integral intensity ratio of the resonance bands at 3.7 and 7.5 to 8.1 ppm, respectively.

Supporting Information Figure S11 shows the GPC elution curves for $pMPC_{98}$ and $pMPC_{296}$ using a phosphate buffer (pH 9) containing 10 vol % acetonitrile as eluent. The GPC elution curves were unimodal with narrow $M_w/M_n$ (=1.05), indicating that the polymerization proceeded in accordance with a living mechanism. However, a marked deviation of $M_n$(GPC) from the theoretical number-average molecular weight ($M_n$(theo)). $M_n$(theo) was observed that can be calculated from

$$M_n(\text{theo}) = \frac{[\text{M}]_0}{[\text{CTA}]_0}\frac{x}{100}\text{MW}_\text{M} + \text{MW}_\text{CTA} \tag{4}$$

where $[M]_0$ and $[CTA]_0$ are concentrations of initial monomer and CTA, respectively, $x$ is conversion, and $MW_M$ and $MW_{CTA}$ are molecular weights of monomer and CTA, respectively. The values for $M_n$(GPC) estimated by GPC are only apparent values probably because sodium poly(styrenesulfonate), a polymer with no bulky side chains compared with pMPC with its bulky phosphorylcholine side chain, was used as a standard for molecular weight calibrations. The DP and $M_n$(NMR) values calculated from $^1$H NMR, and the $M_n$(GPC) and $M_w/M_n$ values estimated from GPC are summarized in Table 1.

Figure 1 shows the $^1$H NMR spectrum for pMPC-*b*-pAaUG3.5 ($P_{98}D_3$) in methanol-$d_4$ at 47 °C. The DP ($=n$) of the pendant dendron-containing block and $M_n$(NMR) of $P_mD_n$ were determined to be 3 and $3.94 \times 10^4$, respectively, based on the integral intensity ratio of the resonance bands of the pendant methylene protons in the pMPC block at 3.7 ppm and the pendant dendron methylene protons at approximately 2.8 ppm. The conversion of AaUG3.5 polymerization using pMPC macro-CTA via RAFT was less than 60% due to the bulky pendant PAMAM Dendron of AaUG3.5. The DP and $M_n$(NMR) values estimated from $^1$H NMR are summarized in Table 1. The block copolymer, pMPC-*b*-pAaUG3.5 was abbreviated as $P_mD_n$. GPC of $P_mD_n$ could not be conducted because the diblock copolymer did not molecularly dissolve in GPC eluent of phosphate buffer containing 10 vol % acetonitrile.

### Association Behavior of Diblock Copolymers Containing Pendant Dendron Groups

Figure 2(a) shows $R_h$ distributions for $P_{98}D_3$ and $P_{296}D_1$, which were obtained from DLS measurements. The $R_h$ values for $P_{98}D_3$ and $P_{296}D_1$ were 156 and 115 nm, respectively, and were estimated from the $R_h$ distributions, indicating that they formed aggregates. Figure 2(b) shows polymer concentration dependence on $R_h$ for $P_{98}D_3$ and $P_{296}D_1$ in water. No dependence of concentration on $R_h$ was observed at concentrations from 0.02 to 0.1 g/L. The relaxation rates (Γ) measured at different scattering angles were plotted as a function of the square of the scattering vector ($q^2$), in Figure 2(c). A linear relation that includes the origin indicates that all of the relaxation modes involved a diffusive process.[44] The end-to-end distances ($L$) of fully extended chains of $P_{98}D_3$ and $P_{296}D_1$ were estimated as 25.3 and 74.3 nm, respectively, based on the counter length of the repeating unit (0.25 nm). The $R_h$ values for $P_{98}D_3$ and $P_{296}D_1$ (156 and 115 nm) were larger than those of the fully extended chain lengths of

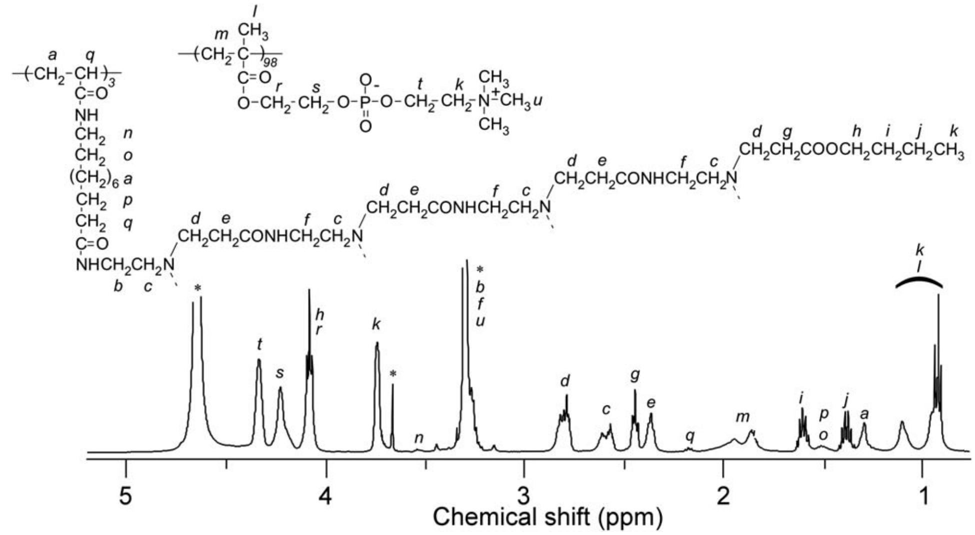



**FIGURE 1** $^1$H NMR spectrum for $P_{98}D_3$ in methanol-$d_4$ at 47 °C.

$P_{98}D_3$ ($L = 25.3$ nm) and $P_{296}D_1$ ($L = 74.3$ nm). These observations suggest that the aggregates were composed of vesicles or large compound micelles.[45] The large compound micelle is probably due to the secondary aggregation of micelles.

Table 2 summarizes the light scattering data for $P_mD_n$ in water. The aggregation number ($N_{agg}$) of the aggregate, defined as the total number of pMPC chains forming one aggregate, can be calculated from the ratio of apparent $M_w$ for the aggregate (estimated from SLS) and the molecular weight for a single polymer chain (unimer) determined from $^1$H NMR ($M_n$(NMR)). The $N_{agg}$ values for $P_{98}D_3$ and $P_{296}D_1$ were 342 and 241, respectively. Hydrophobic interactions of $P_{296}D_1$ may be less common than those of $P_{98}D_3$, because $P_{296}D_1$ has relatively longer hydrophilic and relatively shorter hydrophobic blocks. Therefore, the value of $N_{agg}$ for $P_{296}D_1$ was smaller than that for $P_{98}D_3$. The $R_g/R_h$ ratios for $P_{98}D_3$ and $P_{296}D_1$ were the same (1.10), suggesting that they have a spherical shape.[46–48]

The structures of the aggregates were confirmed by TEM observations, shown in Figure 3. Rugged spherical shapes were observed for the aggregates formed from $P_{98}D_3$. In contrast, hollow spherical shapes were observed for the aggregates formed from $P_{296}D_1$. The block copolymers did not form simple core-shell spherical micelles according to light scattering and TEM data. For $P_{98}D_3$, the polymers may form large compound micelles. However, $P_{296}D_1$ may have formed vesicles, according to TEM observations, which showed a dark domain corresponding to polymers stained with phosphotungstate and a bright domain corresponding to the unstained inner hollow core. Average diameters for the aggregates formed from $P_{98}D_3$ and $P_{296}D_1$ observed in TEM images were 380 and 240 nm, respectively. These values were close to those estimated from light scattering measurements. The $2R_h$ values for aggregates formed from $P_{98}D_3$ and $P_{296}D_1$ were 312 and 230 nm, respectively.[49]

Critical aggregation concentration (cac) for the aggregates in water was determined by a fluorescence technique using *N*-phenyl-1-naphthylamine (PNA) as a fluorescence probe. A decrease in the polarity around PNA leads to a blue shift of its fluorescence emission maximum.[50] Fluorescence spectra of PNA probes dissolved in water in the presence of the diblock copolymers were measured at varying polymer concentrations. Fluorescence emission maxima were plotted as a function of polymer concentration, shown in Figures 4 and 5. The emission maxima were nearly constant at 463 nm in the low $C_p$ region, indicating the absence of hydrophobic domains in aggregates of the block copolymers. The emission maxima exhibited a substantial decrease with increasing $C_p$, suggesting incorporation of PNA molecules into the hydrophobic portion of the polymer aggregates. The hydrophobic portion that can incorporate PNA may associate with the terminal *n*-butyl groups of the pendant dendron groups of the diblock copolymer above the cac value. The emission maximum wavelength for $P_{98}D_3$ and $P_{296}D_1$ decreased with increasing $C_p$ at approximately 0.009 and 0.015 g/L, respectively. The polymer concentration at which the emission maximum began to blue-shift corresponds to cac. Thus, the cac appears to decrease when the DP of hydrophobic dendron-containing blocks increased from 1 to 3. The cac values for $P_{98}D_3$ and $P_{296}D_1$ are listed in Table 2.

### Incorporation of Guest Molecules

Fluorescence measurements were obtained using hydrophilic Texas red-Dex (MW = 3000) as a fluorescence probe to confirm the incorporation of hydrophilic guest polymer molecules into the hollow core of vesicles formed from $P_{296}D_1$.

After 80 h dialysis, hydrophilic Texas red-Dex was removed completely from the inside of the dialysis bag to the outside, confirmed by the lack of fluorescence emission from Texas red-Dex in the experiment performed without $P_{296}D_1$. Texas red-Dex can pass through a dialysis membrane with a 50 nm pore size. In contrast, Texas red-Dex can be incorporated

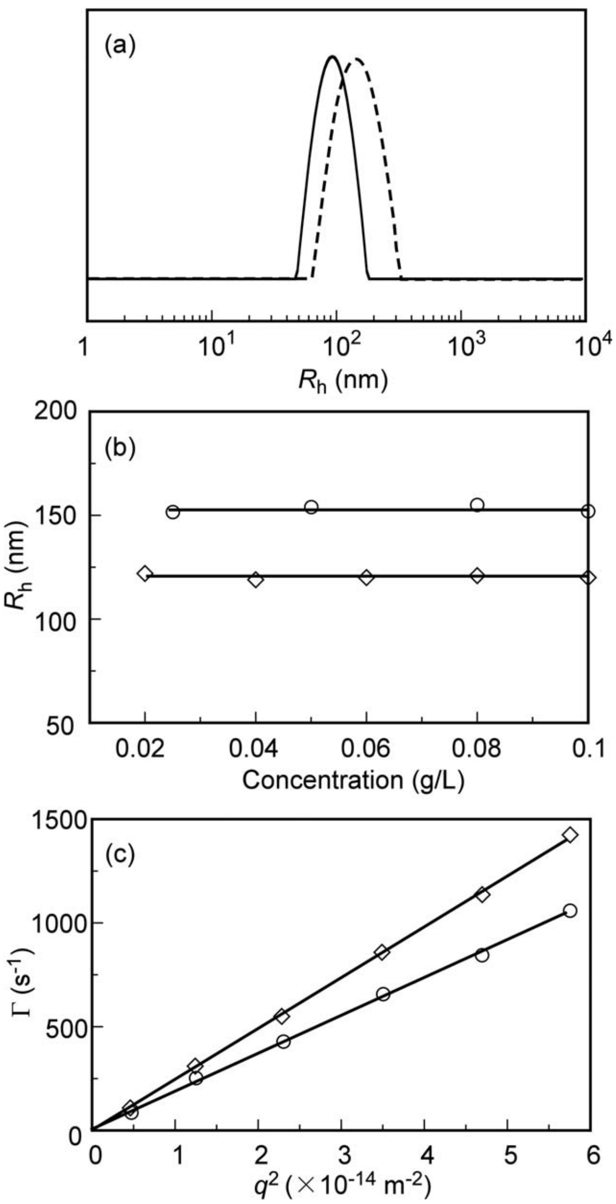



**FIGURE 2** (a) Hydrodynamic radius ($R_h$) for $P_{98}D_3$ (---) and $P_{296}D_1$ (—) in water at a polymer concentration ($C_p$) = 0.1 g/L. (b) $C_p$ dependence on $R_h$ for $P_{98}D_3$ (○) and $P_{296}D_1$ (◇) in water. (c) Angular dependence of $P_{98}D_3$ (○) and $P_{296}D_1$ (◇) in water at $C_p$ = 0.1 g/L.

into the hydrophilic hollow core of vesicles formed from $P_{296}D_1$, indicated by the observation of the Texas red-Dex fluorescence emission. When Texas red-Dex was incorporated into the hydrophilic hollow core of $P_{296}D_1$ vesicles, it could not pass through the dialysis membrane, because of the size of the $P_{296}D_1$ aggregate ($R_g$ = 127 nm). The LC and LE values were calculated using eqs (1) and (2) as 0.34 and 1.69%, respectively. The LE value of rhodamine B by vesicles formed from hydrophobically modified dextran is reported to be 1.80%, which is close to the result found for $P_{296}D_1$.[51] The fluorescence emission from Texas red-Dex was not observed after dialysis using $P_{98}D_3$, which suggests that Texas red-Dex cannot be incorporated into the aggregates formed from $P_{98}D_3$, likely because the $P_{98}D_3$ aggregate may not have a hollow core that accepts hydrophilic Texas red-Dex.

**TABLE 2** Apparent Weight-Average Molecular Weight ($M_w$), Aggregation Number ($N_{agg}$), Hydrodynamic Radius ($R_h$), Radius of Gyration ($R_g$), $R_g/R_h$ Values, and Critical Aggregation Concentration (cac) for the Diblock Copolymers ($P_mD_n$)

| | $M_w \times 10^{-7}$ | $N_{agg}$ | $R_h$ (nm) | $R_g$ (nm) | $R_g/R_h$ | cac (g/L) |
|---|---|---|---|---|---|---|
| $P_{98}D_3$ | 1.35 | 342 | 156 | 172 | 1.10 | 0.009 |
| $P_{296}D_1$ | 2.20 | 241 | 115 | 127 | 1.10 | 0.015 |

Generally, when the length of the hydrophobic block is shorter than that of the hydrophilic block, the amphiphilic diblock copolymers tend to form core-shell spherical micelles in water. However, when the length of the hydrophobic block is greater than that of the hydrophilic block, the polymer tends to form rods or vesicles.[52] For the diblock copolymers ($P_mD_n$), $P_{98}D_3$ formed large compound micelles, since the length of the hydrophilic pMPC block was longer than that of the hydrophobic pAaUG3.5 block. And $P_{296}D_1$ formed vesicles in water, suggesting that the structure of aggregates depends not only on the hydrophobic/hydrophilic balance but also on the chemical architecture of each block.

## CONCLUSIONS

A hydrophobic monomer (AaUG3.5) containing a pendant poly(amido amine) (PAMAM) dendron with 16 *n*-butyl terminal groups and one acrylamide focal point was prepared by alternating repeated Michael additions and ester-amide exchanges. The products for each step of the reaction were confirmed by NMR and IR spectra. AaUG3.5 was polymerized with a well-controlled structure using hydrophilic pMPC macro-CTA ($M_w/M_n$ = 1.05) via RAFT polymerization. The resulting amphiphilic diblock copolymers ($P_mD_n$) contained a biocompatible linear pMPC block and hydrophobic pendant dendron-containing block. Two diblock copolymers with different compositions ($P_{98}D_3$ and $P_{269}D_1$) also were prepared. The self-association behavior of the amphiphilic diblock copolymers in water were examined using DLS and SLS measurements. The $R_h$ values for $P_{98}D_3$ and $P_{269}D_1$ were larger than the end-to-end distances of the fully expanded chains, suggesting that these aggregates were not simple core-shell spherical micelles. Aggregation numbers for $P_{98}D_3$ and $P_{269}D_1$ were 342 and 241, respectively. The critical aggregation concentrations for $P_{98}D_3$ and $P_{269}D_1$ were 0.009 and 0.0015 g/L, respectively. According to TEM and light scattering data, aggregates formed from $P_{98}D_3$ and $P_{269}D_1$ were large compound micelles and vesicles, respectively. The vesicles formed from $P_{269}D_1$ could incorporate hydrophilic guest polymer molecules of dextran with a molecular weight of 3000 into the hollow core. In contrast, the large

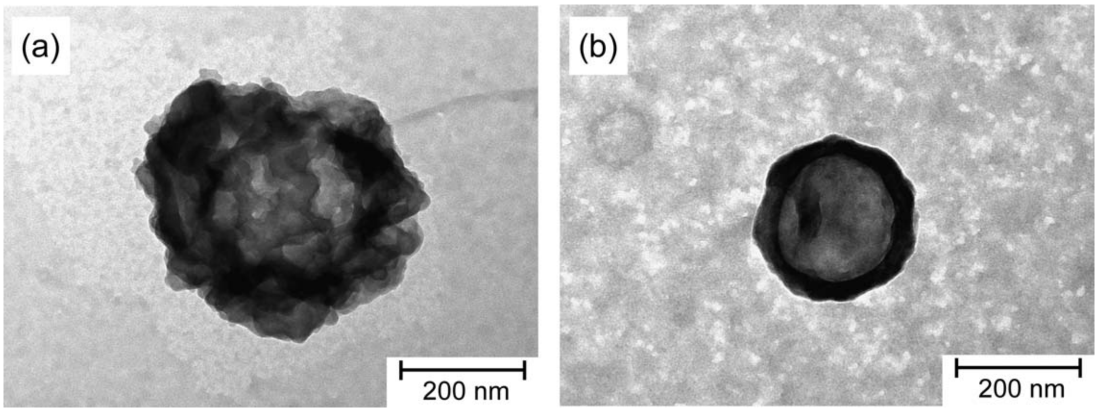



**FIGURE 3** EM images for (a) $P_{98}D_3$ and (b) $P_{296}D_1$ after being stained with sodium phosphotungstate.

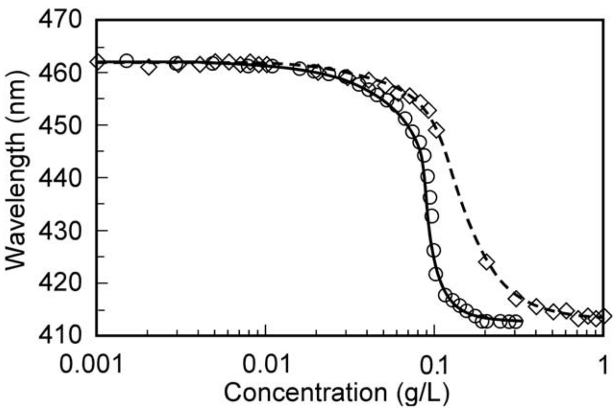



**FIGURE 4** Maximum fluorescence emission wavelength of *N*-phenyl-1-naphthylamine (PNA) plotted against polymer concentrations for $P_{98}D_3$ (○) and $P_{296}D_1$ (◇) in water. Excitation wavelength was 330 nm.

compound micelles formed from $P_{98}D_3$ could not incorporate dextran, because the aggregates did not have a hydrophilic hollow core that could accept hydrophilic guest molecules. The vesicle formed from $P_{269}D_1$ is expected to be a good candidate as a carrier in drug delivery systems, because of the biocompatible pMPC chains covering its surface.

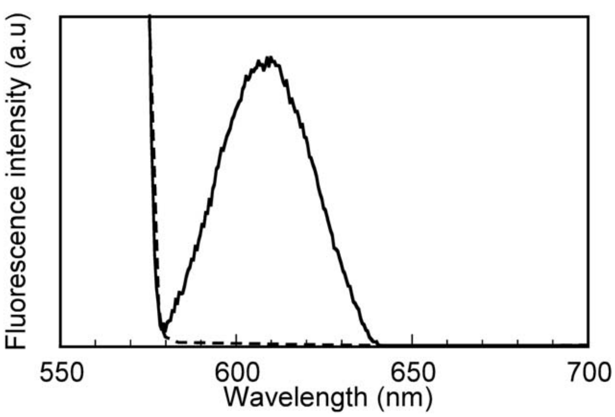



**FIGURE 5** Fluorescence spectra for aq. Texas red-Dex solutions in the presence (-) and absence (---) of $P_{296}D_1$ after dialysis.

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