

# Hydrotropic Dendrimers of Generations 4 and 5: Synthesis, Characterization, and Hydrotropic Solubilization of Paclitaxel

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Polyglycerol dendrimers (PGDs) with 4–5 generations were synthesized and used to investigate the effect of dendritic architecture and its generation on aqueous solubilization of paclitaxel (PTX), a poorly water-soluble drug. Chemical and physical properties of the PGDs were characterized by NMR, MALDI-TOF mass, GPC, viscosity, and dynamic light scattering measurements. The PTX solubility in all the solutions of PGDs, even below 10 wt %, was much higher than that in PEG400 that is commonly used as a cosolvent or a hydrotropic agent. Increase in the PTX solubility by PGDs was dependent on the dendrimer generation. The dendritic structure was the reason for the enhanced solubility of PTX even at low concentrations. <sup>1</sup>H NMR spectra of PTX before and after mixing with PGDs in D<sub>2</sub>O suggested that the aromatic rings and some methyne groups of PTX were surrounded by PGDs. PGDs, which do not require hydrophobic segment as in polymeric micelles, provide an alternative method of hydrotropic solubilization of poorly soluble drugs.

## INTRODUCTION

Formulation of poorly water-soluble drugs has been one of the most important problems in drug delivery. Previous formulations have utilized surfactant and polymeric micelles, micro- or nanoparticles, solid dispersion, complexation with cyclodextrin, and cosolvents (1). Cosolvent methods have been widely used because of easy processing for fabricating oral as well as injectable formulations (2, 3). The U.S. Food and Drug Administration has approved six water-miscible cosolvents: glycerin, poly(propylene glycol), poly(ethylene glycol) (PEG), *N,N*-dimethylformamide, Cremophor, and ethanol (1). Use of such organic solvents is limited because of their toxicities. In commercially available formulations, paclitaxel is formulated in a 50:50 mixture of Cremophor EL (castor oil modified with PEG-35) and ethanol, which is diluted in normal saline or dextrose solution (to make 5 wt % paclitaxel concentration) before administration (4). This formulation can cause fatal hypersensitivity reactions mainly due to cytotoxicity of the amphiphilic compound and the organic solvent (5). For this reason, safer vehicles for delivery of paclitaxel have been explored.

PEGs have been frequently used for increasing the water-solubility of poorly soluble drugs. PEG with molecular weight of 400 g/mol (PEG400) has been used as a cosolvent for various poorly water-soluble drugs (6, 7). PEG400 increases the solubility of  $\beta$ -estradiol 4–5 orders of magnitude when its concentration is 80 wt % and higher (6). The majority of PEG400 are capable of self-association through hydrogen bonding at higher concen-

tration regions (over 80 wt %). Such associations may alter water structure to influence the water-solubility of poorly soluble drugs (8). A high density of PEG400 or ethylene glycol units is the key factor in increasing the solubility of poorly water-soluble drugs.

In our previous studies, graft and star-shaped graft copolymers of PEG400 were examined as solubilizers of paclitaxel (9). These molecular architectures enhanced the aqueous solubility of paclitaxel. In addition to graft and star-shaped graft copolymers of PEG400, polyglycerol dendrimers (PGDs) were examined for their ability to increase the water-solubility of paclitaxel. Well-defined hyperbranched polyglycerols were synthesized by ring-opening multibranching polymerization (10) and used as biocompatible materials (11, 12). PGD with generation 3 was synthesized by step-by-step allylation and dihydroxylation (13). The density of ethylene glycol units in PGDs is expected to be higher than that of linear PEGs. PGDs have a good potential as biomaterials because of their high water solubility, chemical reactivity, and structural similarity to PEG with high density of the ethylene glycol unit. PGDs increased solubilization of paclitaxel more than the graft and star-shaped graft copolymers did. Although the PGDs have a good potential as a drug solubilizer, PGDs with 4 and 5 generations were not characterized, and the mechanism of the solubility enhancement of paclitaxel was not examined. In this study, PGDs with 4 and 5 generations were synthesized and characterized in terms of chemical structure, solution properties, and solubility mechanism of paclitaxel.

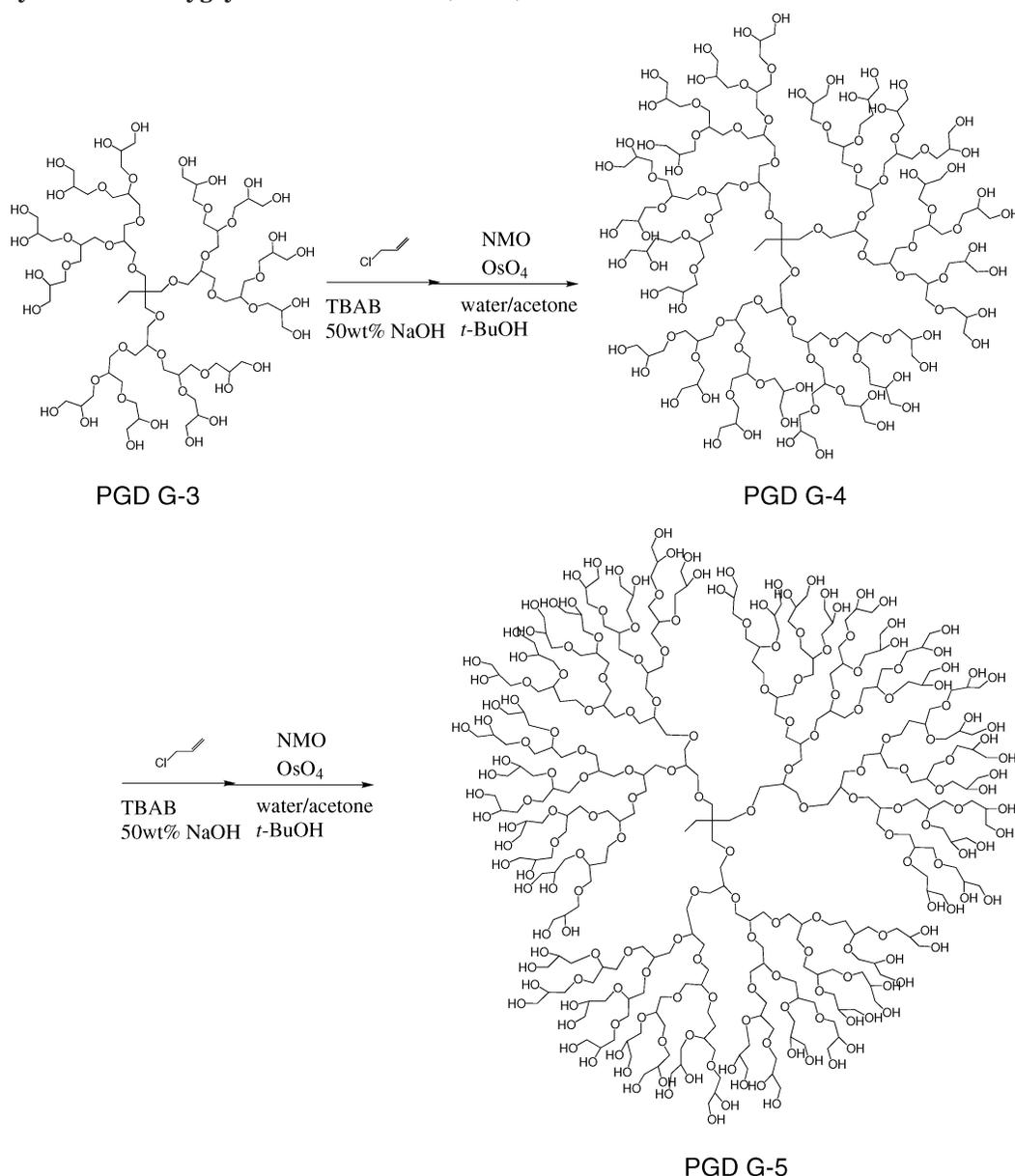
## MATERIALS AND METHODS

**Materials.** Tetrabutylammonium bromide (TBAB), 50 wt % of sodium hydroxide solution, allyl chloride, magnesium sulfate (MgSO<sub>4</sub>), silica gel, *N*-methylmorpholine *N*-oxide (NMO), and aqueous (4 wt %) OsO<sub>4</sub> solution were purchased from Aldrich (Milwaukee, WI) and used without further purification. *t*-Butanol (*t*-BuOH) and

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**Scheme 1. Synthesis of Polyglycerol Dendrimers (PGDs)<sup>a</sup>**

<sup>a</sup> TBAB: tetrabutylammonium bromide, NMO: *N*-methylmorpholine *N*-oxide, OsO<sub>4</sub>: osmium oxide 4 wt % aqueous solution.

*N,N*-dimethylformamide (DMF, HPLC grade) were purchased from Fisher. Benzylated cellulose membrane (MWCO 1000) was purchased from Sigma (St. Louis, MO). Paclitaxel was obtained from Samyang Genex Corp. (Taejeon, South Korea). Acetone, petroleum ether, ethyl acetate, toluene, and methanol were of a reagent grade.

**Synthesis of Polyglycerol Dendrimers (PGDs) Started from Generation 3 (Scheme 1).** PGDs (generations 4 and 5) were synthesized according to the report of Haag et al. (13). Each generation of the dendrimer was synthesized by a two-step process based on allylation of alcohols and catalytic dihydroxylation. A part of the purification process was modified.

*PGD with Generation 3 (PGD G-3).* PGD G-3 was synthesized in the same method of Haag et al. (13). <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): 4.85 (s, OH), 3.90–3.31 (m, 105H), 3.31 (s, 6H, C(CH<sub>2</sub>)<sub>3</sub>), 1.42 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 0.89 (t, *J* = 7.20 Hz, 3H, CH<sub>3</sub>); ESI-MS (4.52 kV, positive) calcd for C<sub>69</sub>H<sub>140</sub>O<sub>45</sub> 1688.9, found 1689.5, 1711.9 (+ Na).

*Allylated PGD with Generation 3 (PGD G-3.5).* In a three-neck flask, PGD G-3 (10.1 g, 5.98 mmol, OH

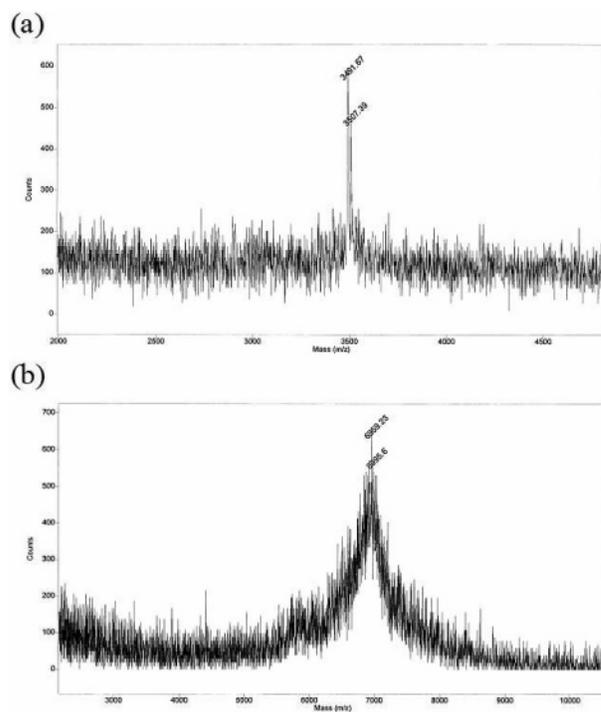
groups: 143.5 mmol) was dissolved in 50 wt % of sodium hydroxide (57 mL) with heating. To the solution, TBAB (4.6 g, 14.3 mmol) was added and dispersed under vigorous stirring using a mechanical stirrer. Then, allyl chloride (75.7 mL, 930 mmol) was added over 20 h at 45 °C under vigorous stirring using the mechanical stirrer. After the addition of 143 mL of toluene to the mixture, the organic phase was separated from the other phase, dried over MgSO<sub>4</sub>, filtered, and concentrated using a rotary evaporator. The crude was further purified by column chromatography (silica gel, petroleum ether/ethyl acetate 10:1 to 1:1) to obtain colorless oil. Oxygen was removed by flowing N<sub>2</sub> gas during the whole process. Yield: 11.1 g, <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): 5.88 (m, 24H, CH=CH<sub>2</sub>), 5.18 (m, 48H, CH=CH<sub>2</sub>), 4.14 (d, *J* = 5.40 Hz, 24H, CHOCH<sub>2</sub>CH=CH<sub>2</sub>), 3.99 (d, *J* = 5.40 Hz, 24H, CH<sub>2</sub>OCH<sub>2</sub>CH=CH<sub>2</sub>), 3.67–3.39 (m, 105H, CH<sub>2</sub>CH(OR)CH<sub>2</sub>O), 3.26 (s, 6H, C(CH<sub>2</sub>)<sub>3</sub>), 1.38 (q, 2H, CH<sub>3</sub>CH<sub>2</sub>), 0.84 (t, *J* = 7.21 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): 135.7 (CHOCH<sub>2</sub>CH=CH<sub>2</sub>), 135.2 (CH<sub>2</sub>OCH<sub>2</sub>CH=CH<sub>2</sub>), 117.1 (CH<sub>2</sub>OCH<sub>2</sub>CH=CH<sub>2</sub>), 116.9 (CHOCH<sub>2</sub>CH=CH<sub>2</sub>), 78.0

(CH<sub>2</sub>CH(OR)CH<sub>2</sub>), 77.6 (CH<sub>2</sub>CH(OCH<sub>2</sub>CH=CH<sub>2</sub>)CH<sub>2</sub>), 72.6 (CH<sub>2</sub>CH(OR)CH<sub>2</sub>), 72.5 (CH<sub>2</sub>OCH<sub>2</sub>CH=CH<sub>2</sub>), 71.6 (CH<sub>2</sub>OCH<sub>2</sub>CH=CH<sub>2</sub>), 70.7 (CHOCH<sub>2</sub>CH=CH<sub>2</sub>), 43.6 (C(CH<sub>2</sub>)<sub>4</sub>), 23.6 (CH<sub>2</sub>CH<sub>3</sub>), 8.3 (CH<sub>2</sub>CH<sub>3</sub>); ESI-MS (4.52 kV, positive) calcd for C<sub>141</sub>H<sub>236</sub>O<sub>45</sub> 2649.6 found 2649.4.

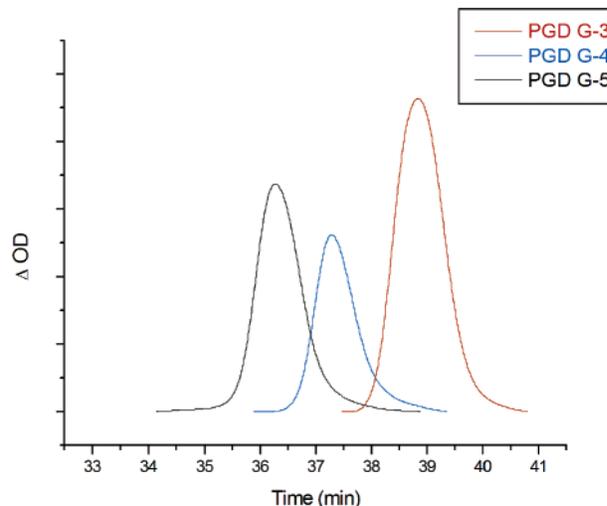
**PGD with Generation 4 (PGD G-4).** In a one-neck flask, PGD G-3.5 (10.9 g, 5.42 mmol, 130 mmol allyl equivalents) and *N*-methylmorpholine *N*-oxide (NMO) (16.8 g, 143 mmol) were added to a mixture of acetone (65 mL), distilled water (65 mL), and *t*-BuOH (13 mL). Aqueous (4 wt %) OsO<sub>4</sub> solution (2.6 mL) was added and stirred for 20 h at room temperature. Then, all volatile compounds were removed in vacuo. The crude products were further purified by dialysis in methanol using benzylated cellulose membrane (MWCO 1000). The solution was concentrated in vacuo, and water was added to the obtained pale yellow oils. Water-insoluble impurity was removed by passing through a microfilter (pore size: 0.45 μm). Finally, the solute was lyophilized to obtain pure PGD G-4 as pale yellow oils. Yield: 11.7 g, <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): 4.85 (s, OH), 3.89–3.42 (m, 231H), 3.35 (s, 6H, C(CH<sub>2</sub>)<sub>3</sub>), 1.44 (q, 2H, CH<sub>2</sub>CH<sub>3</sub>), 0.92 (t, *J* = 6.92 Hz, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD): 80.3 ((CH<sub>2</sub>)<sub>2</sub>-CH<sub>2</sub>C(H)OR), 74.5 (CH<sub>2</sub>OCH<sub>2</sub>CH(OH)CH<sub>2</sub>OH), 73.4 (C(CH<sub>2</sub>)<sub>3</sub>), 72.9 (ROCH<sub>2</sub>C(H)OR)CH<sub>2</sub>OR), 72.6 (CHOCH<sub>2</sub>-CH(OH)CH<sub>2</sub>OH), 71.7 (CHOCH<sub>2</sub>CH(OR)CH<sub>2</sub>OR), 64.9 (CH<sub>2</sub>OH), 45.1 (C(CH<sub>2</sub>)<sub>4</sub>), 25.2 (CH<sub>2</sub>CH<sub>3</sub>), 9.0 (CH<sub>2</sub>CH<sub>3</sub>); MALDI-TOF MS calcd for C<sub>144</sub>H<sub>290</sub>O<sub>93</sub> 3507.8 found 3507.4 +H, 3491.7 -H<sub>2</sub>O.

**Allylated PGD with Generation 4 (PGD G-4.5).** In a three-neck flask, PGD G-4 (8.3 g, 2.63 mmol, OH groups: 126.2 mmol) was dissolved in 50 wt % of sodium hydroxide (45 mL) with heating (viscous gum was sometimes observed). To the solution, TBAB (3.65 g, 11.3 mmol) was added and dispersed under vigorous stirring using a mechanical stirrer. Then, allyl chloride (64.4 mL, 791 mmol) was added over 20 h at 45 °C under vigorous stirring using the mechanical stirrer. After the addition of 126 mL of toluene to the mixture, the organic phase was separated from the other phase, dried over MgSO<sub>4</sub>, filtered, and concentrated using a rotary evaporator. The crude was further purified by column chromatography (silica gel, petroleum ether/ethyl acetate 10:1 to 1:1) to obtain colorless oil. Oxygen should be removed via flowing N<sub>2</sub> gas during the whole process. Yield: 5.84 g, <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): 5.90 (m, 48H, CH=CH<sub>2</sub>), 5.24 (m, 96H, CH=CH<sub>2</sub>), 4.15 (d, *J* = 5.10 Hz, 48H, CHOCH<sub>2</sub>CH=CH<sub>2</sub>), 4.00 (d, *J* = 5.10 Hz, 24H, CH<sub>2</sub>OCH<sub>2</sub>-CH=CH<sub>2</sub>), 3.67–3.30 (m, 231H, CH<sub>2</sub>CH(OR)CH<sub>2</sub>O), 3.33 (brs, 6H, C(CH<sub>2</sub>)<sub>3</sub>), 1.44 (brq, 2H, CH<sub>3</sub>CH<sub>2</sub>), 0.86 (brt, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>): 135.7 (CHOCH<sub>2</sub>CH=CH<sub>2</sub>), 135.3 (CH<sub>2</sub>OCH<sub>2</sub>CH=CH<sub>2</sub>), 117.2 (CH<sub>2</sub>OCH<sub>2</sub>CH=CH<sub>2</sub>), 117.1 (CHOCH<sub>2</sub>CH=CH<sub>2</sub>), 77.9 (CH<sub>2</sub>CH(OR)CH<sub>2</sub>), 77.5 (CH<sub>2</sub>CH(OCH<sub>2</sub>CH=CH<sub>2</sub>)CH<sub>2</sub>), 72.7 (CH<sub>2</sub>CH(OR)-CH<sub>2</sub>), 72.7 (CH<sub>2</sub>OCH<sub>2</sub>CH=CH<sub>2</sub>), 71.7 (CH<sub>2</sub>OCH<sub>2</sub>CH=CH<sub>2</sub>), 70.7 (CHOCH<sub>2</sub>CH=CH<sub>2</sub>), 43.4 (C(CH<sub>2</sub>)<sub>4</sub>), 23.5 (CH<sub>2</sub>CH<sub>3</sub>), 8.2 (CH<sub>2</sub>CH<sub>3</sub>).

**PGD with Generation 5 (PGD G-5).** In a one-neck flask, PGD G-4.5 (5.84 g, 1.1 mmol, 52.8 mmol allyl equivalents) and *N*-methylmorpholine *N*-oxide (NMO) (6.72 g, 57.2 mmol) were added to a mixture of acetone (26 mL), distilled water (26 mL), and *t*-BuOH (5.2 mL). Aqueous (4 wt %) OsO<sub>4</sub> solution (1.1 mL) was added and stirred for 20 h at room temperature. Then, all volatile compounds were removed in vacuo. The crude products were further purified by dialysis in methanol using benzylated cellulose membrane (MWCO 1000). The solution was concentrated in vacuo, and water was added to the obtained pale yellow oils. Water-insoluble impurity was



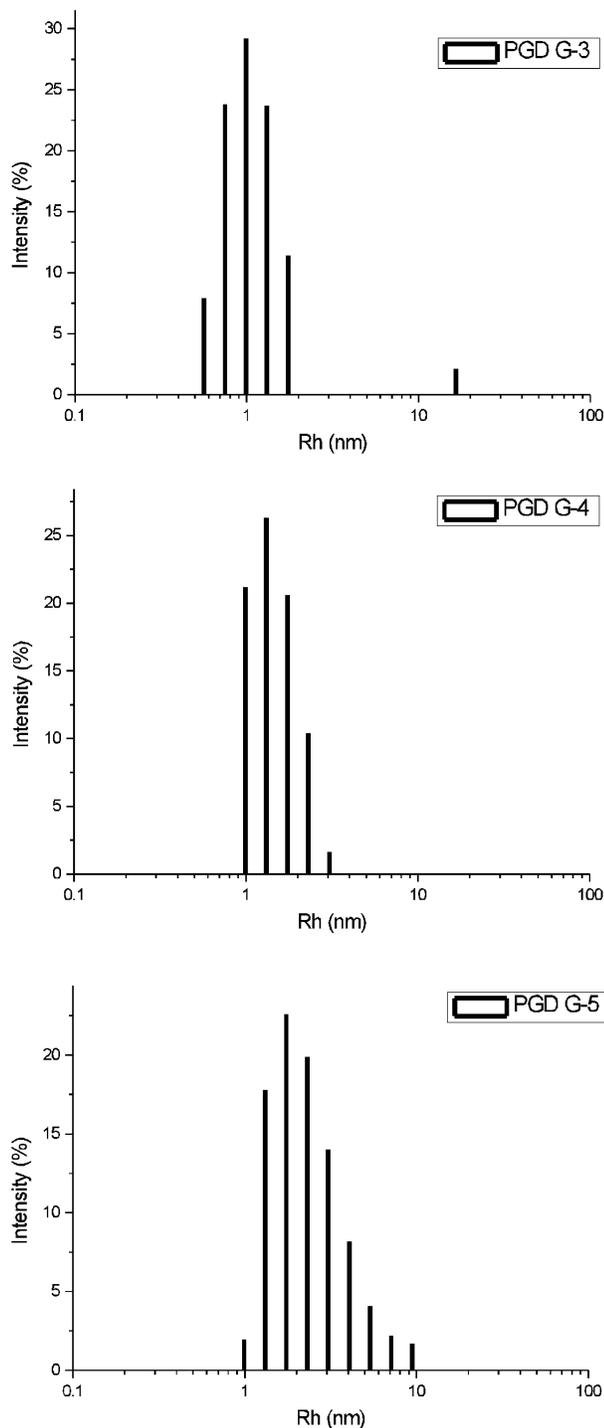
**Figure 1.** MALDI-TOF mass spectra of (a) PGD G-4 and (b) PGD G-5.



**Figure 2.** GPC charts of PGDs (G-3, G-4, and G-5). Eluent: DMF; flow rate: 0.6 mL/min; detector: reflective index.

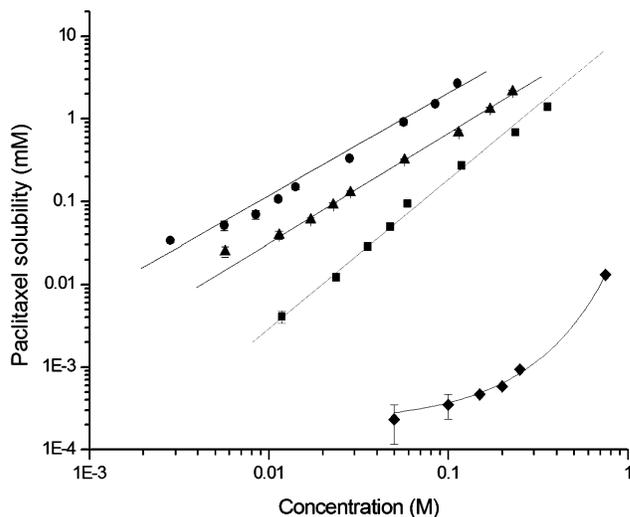
removed by passing through a microfilter (pore size: 0.45 μm). Finally, the solute was lyophilized to obtain pure PGD G-5 as pale yellow oils. Yield: 6.1 g, <sup>1</sup>H NMR (300 MHz, CD<sub>3</sub>OD): 4.85 (s, OH), 3.79–3.42 (m, 477H), 3.35 (s, 6H, C(CH<sub>2</sub>)<sub>3</sub>), 1.45 (brq, 2H, CH<sub>2</sub>CH<sub>3</sub>), 0.94 (brt, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD): 80.3 ((CH<sub>2</sub>)<sub>2</sub>-CH<sub>2</sub>C(H)OR), 74.4 (CH<sub>2</sub>OCH<sub>2</sub>CH(OH)CH<sub>2</sub>OH), 73.4 (C(CH<sub>2</sub>)<sub>3</sub>), 72.9 (ROCH<sub>2</sub>C(H)OR)CH<sub>2</sub>OR), 72.6 (CHOCH<sub>2</sub>-CH(OH)CH<sub>2</sub>OH), 71.7 (CHOCH<sub>2</sub>CH(OR)CH<sub>2</sub>OR), 64.9 (CH<sub>2</sub>OH), 45.1 (C(CH<sub>2</sub>)<sub>4</sub>), 25.2 (CH<sub>2</sub>CH<sub>3</sub>), 9.0 (CH<sub>2</sub>CH<sub>3</sub>); MALDI-TOF MS calcd for C<sub>291</sub>H<sub>584</sub>O<sub>189</sub> 7103.6 found 6769.2 +H, 6959.2 -H.

**Characterization of PGDs.** *NMR Spectroscopy.* <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained on a Bruker ARX300 spectrometer at 300 and 75 MHz, respectively. For the analysis of the interaction between PGDs and paclitaxel in a solubilized state in D<sub>2</sub>O, a 750 MHz NMR apparatus (Varian, Unity plus, CA) was used.

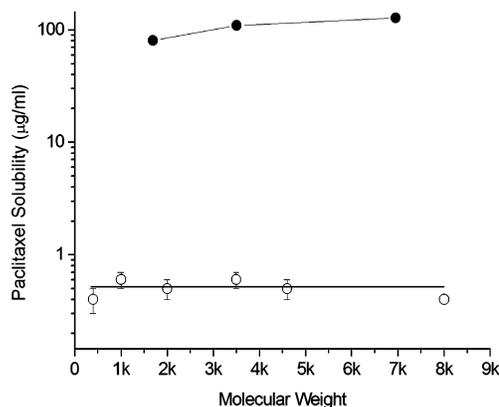


**Figure 3.** Regularization histograms of PGDs on DLS. (a) G-3, (b) G-4, and (c) G-5.

**Mass Spectroscopy.** Electrospray ionization mass spectrometry (ESI-MS) assay was carried out on a Finnigan-MAT LCQ (ThermoFinnigan Corp, San Jose, CA). The electrospray needle voltage was set at 4.5 kV, the heated capillary voltage was set to 10 V, and the capillary temperature was set to 225 °C. Typical background source pressure was  $1.2 \times 10^{-5}$  Torr as read by an ion gauge. The sample flow rate was approximately 10  $\mu\text{L}/\text{min}$ . The drying gas was nitrogen. The LCQ was scanned to 2000 amu for these experiments. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry was carried out using a PerSeptive Biosystems Voyager mass spectrometer (Framingham, MA) in linear mode. Samples were prepared by casting the matrix compound (2,5-



**Figure 4.** Aqueous paclitaxel solubility as a function of the PGD concentration (mean  $\pm$  SD,  $n = 3$ ). (circle) G-5, (triangle) G-4, (square) G-3, and (diamond) PEG400.



**Figure 5.** Dependence of molecular weight of PGDs (closed circle) and PEG (open circle) on the aqueous paclitaxel solubility. The concentration of PGDs and PEG was 10 wt%.

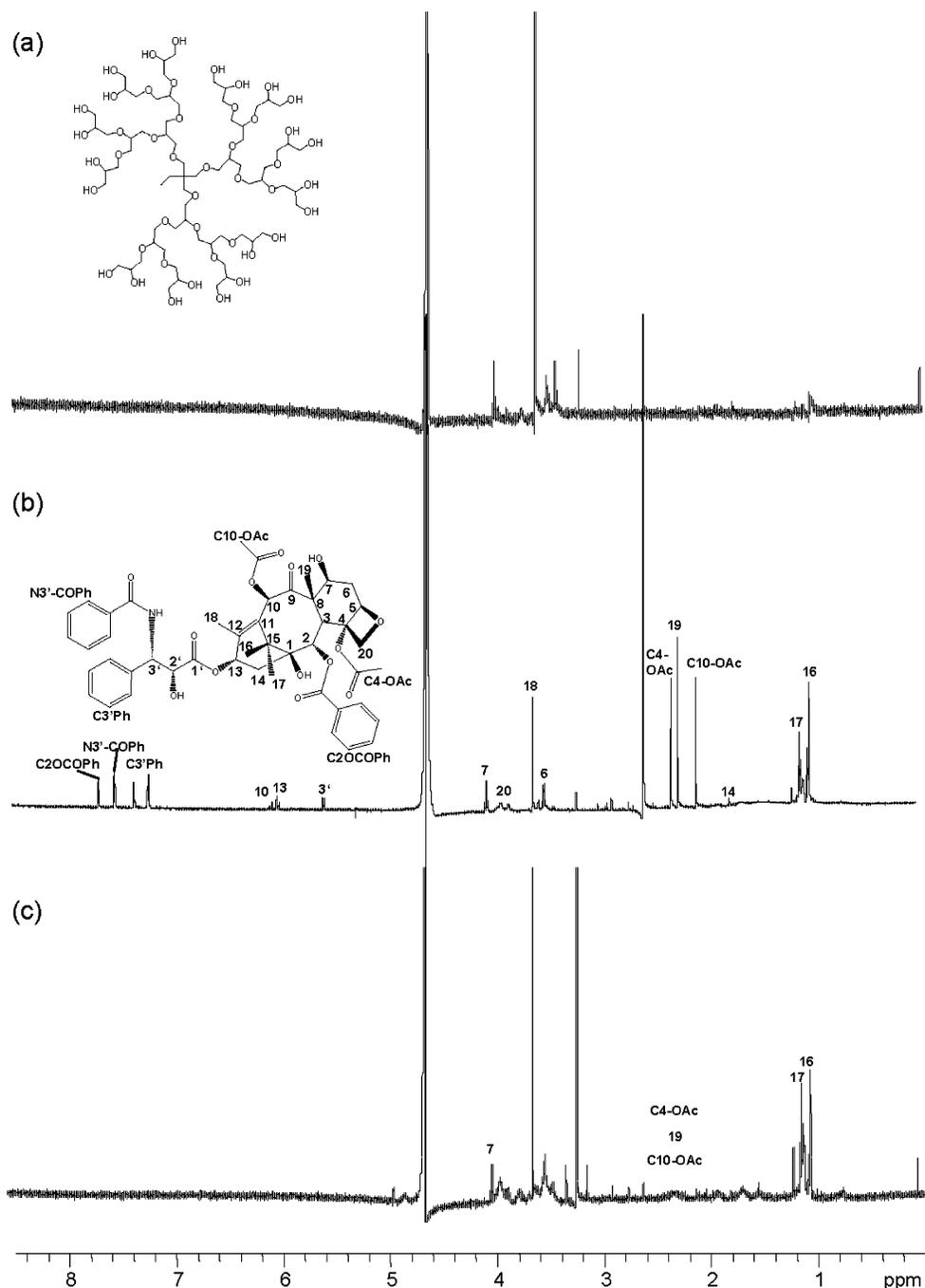
dihydroxyacetophenone) with PGDs ( $\sim 0.2$  mg/mL in methanol) onto the slide, and the solvent was evaporated. Ionization was accelerated with 20 kV in the positive ion mode.

**Gel permeation Chromatography (GPC).** The molecular weight and molecular weight distribution were analyzed by GPC equipped with an Agilent 1100 series RI detector, quaternary pump, and PL aquagel-OH columns with pore sizes of 30, 40, and 50 Å (Agilent Technologies, Wilmington, DE). The eluent was DMF, and flow rate was 0.6 mL/min. The molecular weights are calibrated with poly(ethylene oxide) standards.

**Dynamic Light Scattering (DLS) Measurements.** Hydrodynamic radius ( $R_h$ ) of the PGDs was measured by DLS using a DynaPro 99 (Protein Solutions, Inc.) at a fixed angle ( $90^\circ$ ). The wavelength of the laser source was 826 nm. The membrane filter with Watman Anotop 10 attachment and the pore diameter 20 nm was used, and 0.15 mL of PGD-dissolved 0.2 M NaCl aqueous solution was injected into a cell. Solution concentration was 1 wt %.

**Viscosity Measurements.** The viscosity of PGDs in water was measured at 25 °C using a Cannon-Manning Semi-Micro Viscometer (Size: 50 C286, Cannon Instrument Co., PA). Specific viscosity ( $\eta_{sp}$ ) of PGDs was calculated by the following equation:

$$\eta_{sp} = (\eta - \eta_0)/\eta_0 = (t - t_0)/t_0$$



**Figure 6.**  $^1\text{H}$  NMR spectra measured using a 750 MHz NMR apparatus in  $\text{D}_2\text{O}$ . (a) PGD G-3 ( $0.35 \mu\text{M}$ ), (b) paclitaxel ( $0.35 \mu\text{M}$ ), and (c) mixture of PGD G-3 and paclitaxel.

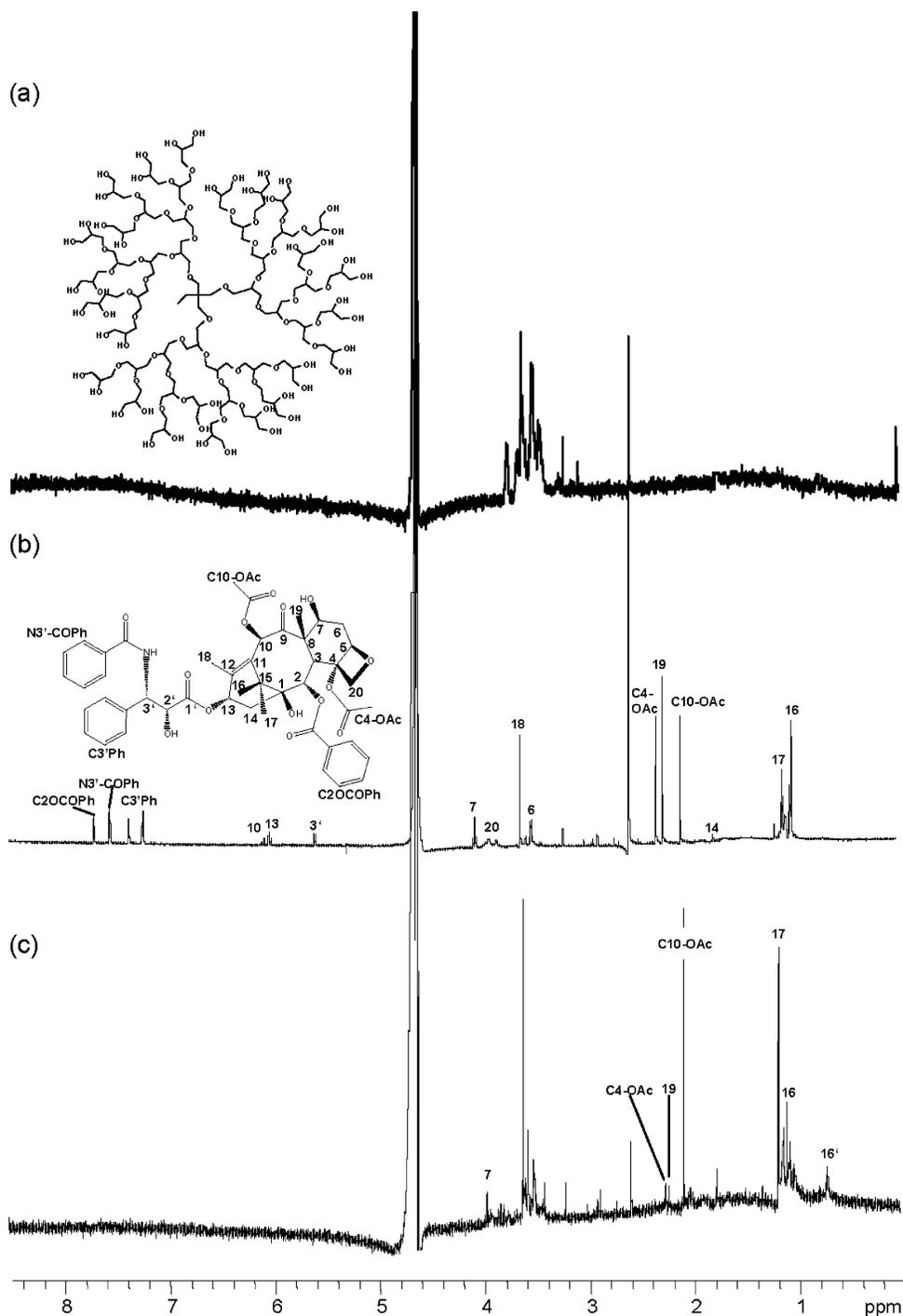
where  $\eta$  is the solution viscosity,  $\eta_0$  the viscosity of water,  $t$  the measuring time of the solution, and  $t_0$  the measuring time of water. From Huggins plot,  $[\eta]$  of PGDs was determined.

**Solubility Test of Paclitaxel.** Excess paclitaxel ( $\sim 10 \text{ mg/mL}$ ) was added to screw-capped vials containing fixed volume of PGD solutions. This mixture in the vials was stirred using a magnetic stirring bar at  $37^\circ\text{C}$ . The samples were taken after 24 h, filtered through a  $0.2 \mu\text{m}$  nylon membrane, and analyzed for paclitaxel using HPLC. The concentration of paclitaxel was determined by an isocratic reverse-phase HPLC (Agilent 1100 series, Agilent Technologies, Wilmington, DE) using a Symmetry column (Water Corp., Milford, MA) at  $25^\circ\text{C}$ . The mobile phase consisted of acetonitrile–water (45:55 v/v) with a flow rate of  $1.0 \text{ mL/min}$ . A diode array detector was set at  $227 \text{ nm}$  and linked to ChemStation software

for data analysis. The paclitaxel concentrations in the samples were obtained from a calibration curve.

## RESULTS AND DISCUSSION

**Synthesis and Structural Characterization of PGD G-4 and G-5.** The obtained PGDs were characterized by NMR, mass, GPC, DLS, and viscosity measurements (Table 1). The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of PGD G-3.5 and G-4.5 were attributed to allyl group [e.g., 5.88 ( $\text{CH}=\text{CH}_2$ ), 5.18 ( $\text{CH}=\text{CH}_2$ ) on the  $^1\text{H}$  NMR spectra, and 135.2 ( $\text{CH}_2\text{OCH}_2\text{CH}=\text{CH}_2$ ), 116.9 ( $\text{CHOCH}_2\text{CH}=\text{CH}_2$ ) on the  $^{13}\text{C}$  NMR spectra]. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of PGD G-4 and G-5 were also attributed to the monomer units. The  $M_w$  of the G-4 and G-5 dendrimers were determined by MALDI TOF-MS. As shown in Figure 1a, two peaks were observed at  $m/z$  3507.4 +H, 3491.7 –H $_2\text{O}$ , indicating that successful synthesis of PGD G-4. As for



**Figure 7.**  $^1\text{H}$  NMR spectra measured using a 750 MHz NMR apparatus in  $\text{D}_2\text{O}$ . (a) PGD G-4 ( $0.35\ \mu\text{M}$ ), (b) paclitaxel ( $0.35\ \mu\text{M}$ ), and (c) mixture of PGD G-4 and paclitaxel.

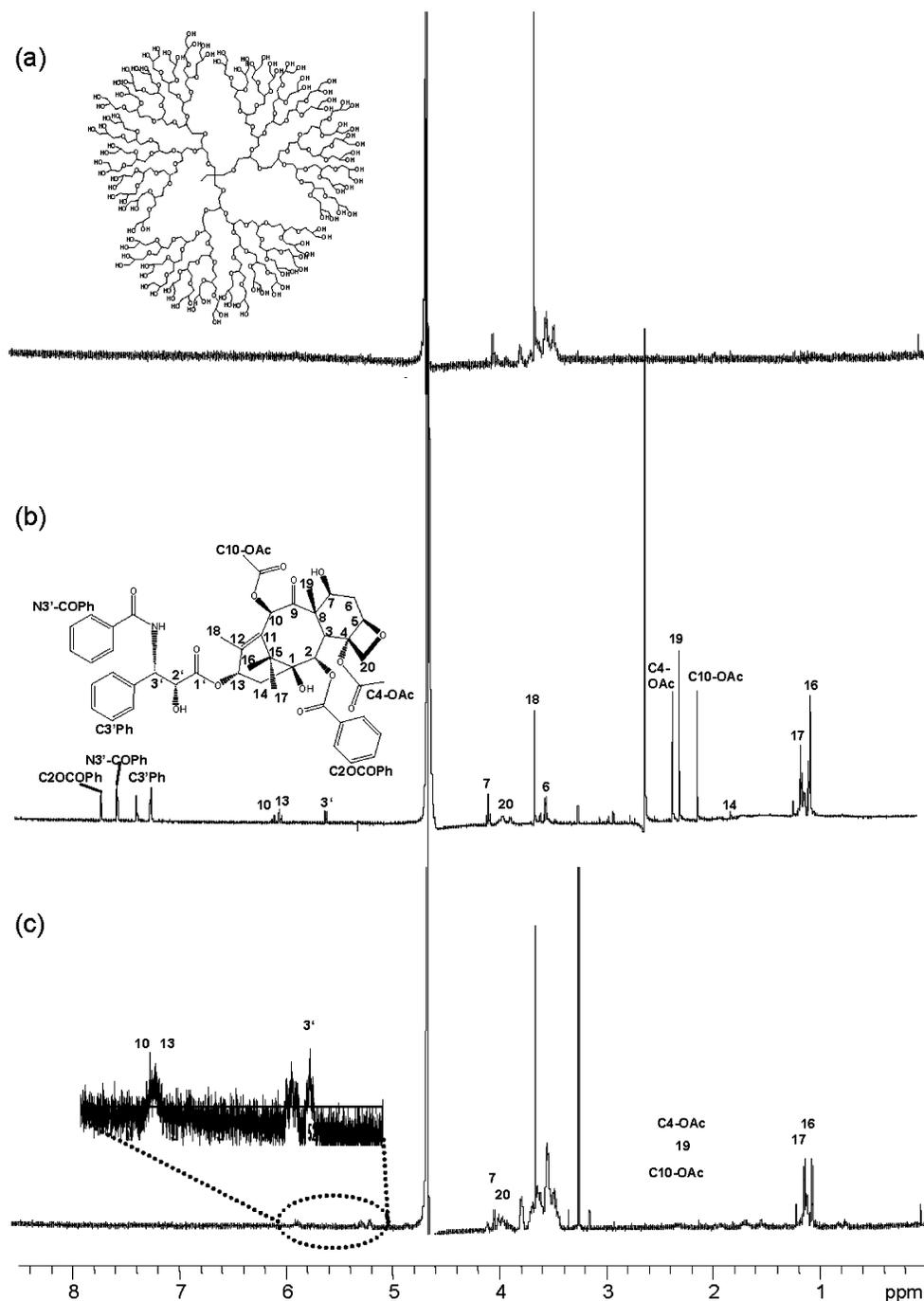
**Table 1. Results of MALDI-MS, GPC, DLS, and Viscosity of PGDs**

sample code	MALDI		GPC			DLS	$[\eta]$ ( $\text{mL}\cdot\text{g}^{-1}$ )
	$M_{\text{theo}}^a$	$M_w^b$	$M_w^c$	$M_n^c$	$M_w/M_n$	$R_h$ (nm)	
PGD G-3	1689	1690	2309	2356	1.02	1.1	1.89
PGD G-4	3508	3507	3764	3841	1.02	1.9	3.90
PGD G-5	7104	6959	5312	5450	1.03	2.4	3.06

<sup>a</sup> Theoretical molecular weight. <sup>b</sup> Determined by MALDI-TOFF mass measurements. <sup>c</sup> Determined by GPC using DMF as an eluent with calibration curve of poly(ethylene oxide) standards.

the PGD G-5, two peaks were observed at  $6769.2 +\text{H}$ ,  $6959.2 -\text{H}$  (Figure 1b). These values were smaller than the calculated value (7103.6), so that there was structural

defect. Steric hindrance of crowded hydroxyl groups of PGD G-4 is thought to be the reason for decreased conversion of allylation and/or dihydroxylation. The molecular weight and molecular weight distribution ( $M_w/M_n$ ) were also analyzed by GPC. As shown in Figure 2, retention time of the PGDs on the GPC charts became shorter with increasing the generation. This order was consistent with the results of mass spectroscopy. As expected,  $M_w/M_n$  was close to 1.00, indicating that the molecular weight distribution of these PGDs was very narrow. The observed molecular weights of G-3, G-4, and G-5 were found to be larger, a little bit larger, and smaller, respectively, than the calculated values. These results, along with MALDI mass, indicate that the PGDs



**Figure 8.** <sup>1</sup>H NMR spectra measured using a 750 MHz NMR apparatus in D<sub>2</sub>O. (a) PGD G-5 (0.35 μM), (b) paclitaxel (0.35 μM), and (c) mixture of PGD G-5 and paclitaxel.

became compact with increasing the generation. This compacted state of dendrimers was consistent with starburst dendrimers (14). Hydrodynamic radius ( $R_h$ ) of the PGDs was measured by DLS. As shown in Figure 3, the regularization histograms indicate very narrow distribution of those  $R_h$  values. The mean  $R_h$  values of PGD G-3, G-4 and G-5, calculated by cumulant analysis, were ca. 1.1, 1.9 and 2.4 nm, respectively.  $[\eta]$  values of PGD G-3, G-4 and G-5 were 1.89, 3.90 and 3.06 mL/g, respectively. Since  $[\eta]$  value of PEG 400 was 4.36 (data not shown), the PGDs seemed to behave as low-molecular-weight compounds in single molecule state in water. These results were also consistent with the GPC results that suggest the compact state of PGDs.

#### The Effect of PGD G-4 and G-5 on Paclitaxel Solubility.

The effect of PGD G-4 and G-5 on paclitaxel

solubilization in water was examined by measuring the concentration of solubilized paclitaxel using HPLC. Paclitaxel solubilities in all the solutions of PGDs (including G-3) were much higher than those of PEG400 (Figure 4). Since the molecular weights of PGDs were much higher than PEG400, the molecular weight should be considered as one of the important factors. To estimate the effect of molecular weight and the dendritic structure, paclitaxel solubilities in various PEGs with different molecular weights were measured and compared with those in PGD solutions (Figure 5). The paclitaxel solubilities in 10 wt % of PEG ( $M_n$ : 400 ~ 8000) aqueous solutions were found to be 0.4–0.6 μg/mL, which was similar to the paclitaxel solubility in water (0.3 μg/mL) (15). On the other hand, the paclitaxel solubilities in 10 wt % of PGDs ( $M_n$ : 1690–6960) were 80–128 μg/mL,

which were 3 orders of magnitude higher than that in pure water. These results suggest that the increased solubility is due to the dendritic architecture. Since the concentration of PGDs required for solubilization of paclitaxel was 30–5000 times higher than the concentration of paclitaxel (Figure 4), it is likely that paclitaxel might not be incorporated into the dendrimer core and PGDs provide hydrotropic effect by providing local high concentrations of ethylene glycol units (16). The ability to enhance the paclitaxel solubility was in the order of G-5 > G-4 > G-3, which is understandable from the hydrotropic effect of dendrimers.

**Proposed Mechanism of the Hydrotropic Solubilization.** The hydrotropic solubilization of paclitaxel by the PGDs was examined in more detail. Since  $^1\text{H}$  NMR data provide valuable information on direct interactions between dendrimer and hydrophobic compounds (17), the chemical shifts of paclitaxel in  $\text{D}_2\text{O}$  before and after mixing with PGD G-3, G-4, and G-5 were examined (Figures 6–8). The 750 MHz NMR apparatus made us to obtain the spectra of paclitaxel even in the lower concentration in  $\text{D}_2\text{O}$  ( $0.35 \mu\text{M}$ ). The measurements at much higher concentration of paclitaxel in the presence of PGD G-4 (Figure 4) were tried, but several hundred times higher concentration of PGD G-4 hindered observing the spectra of paclitaxel. Therefore, the concentration of paclitaxel was fixed to be maximum concentration ( $0.35 \mu\text{M}$ ), and the same molar amounts of PGDs G-3, G-4, and G-5 were dissolved in the solution to observe the chemical shifts. Before mixing paclitaxel with PGDs, the peaks of all the aromatic rings (C2OCOPh, N3'-COPh, and C3'-Ph) and methyne groups of C10, C13, and C3' were observed at 7.69, 7.53, 7.35–7.21, 6.05, 6.01, and 5.94, respectively (Figures 6–8b). When mixing with the same concentration of PGDG-3, those peaks of all the aromatic rings, methyne groups were suppressed (Figure 6c). In addition, the peaks of C4-OAc (2.25 ppm), methyl of C19 (2.21 ppm) and C10-OAc (2.06 ppm) in Figure 6b were broadened (Figure 6c). Similar tendency of the peak suppression has been reported in the case of hydrophilic-hydrophobic block copolymers (18) and dendrimers (19): the peaks of hydrophobic parts were suppressed in  $\text{D}_2\text{O}$  when the polymers form micelles with core-shell structure. Thus, the results suggest that the aromatic rings, methyne groups, and acetyl groups of paclitaxel are surrounded by the PGD G-3. The methyl peaks of C17 and 16 (1.11–1.0 ppm) were clearly observed after the mixing, suggesting that the whole paclitaxel molecules could not be incorporated in the PGD core. Similar tendency in the case of PGD G-4 was observed, but the peaks of C7 methyne (3.99 ppm), C4-OAc (2.25 ppm), methyl of C19 (2.21 ppm), C10-OAc (2.06 ppm), and the methyl peaks of C17 and 16 were observed after the mixing, as shown in Figure 7c. Interestingly, when mixing with PGD G-5, the methyne peaks of C10, C13, and C3' were observed with some chemical shift change to higher magnetic fields (Figure 8c). These results suggest that the way of intermolecular interactions for the hydrotropic solubilization is changeable with increasing the generation of PGDs. Presumably, the increased number of hydroxyl groups participates hydrogen bonds with amide, ester, and/or hydroxyl group of paclitaxel. Since PGDs did not have any hydrophobic core like the dendritic unimolecular micelles composed of many aromatic rings (19), the solubilization ability without using the hydrophobic region (such as aromatic rings) suggests a new mechanism of solubilization. Our ongoing study shows that the solubilized paclitaxel is amorphous, but

the chemical structure was stable in the solubilized states over several months.

## CONCLUSION

Polyglycerol dendrimers (PGDs) with 4–5 generations were synthesized and characterized by NMR, MALDI-TOF mass, GPC, viscosity, and dynamic light scattering measurements. Polydispersity of the obtained PGDs was very narrow, and the PGDs in water exist in compacted states. The paclitaxel solubility was increased with the higher generation of PGD, and the effect was due to the dendritic architecture. PGDs increased the paclitaxel solubility by surrounding aromatic rings and some methyne groups of paclitaxel. The hydrotropic solubilization by PGDs does not require a hydrophobic segment as in polymeric micelles, and it provides an alternative approach of increasing the solubility of poorly soluble drugs.

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