A thermoresponsive amphiphilic dendron — Synthesis, characterization, and self-assembled micelles for controlled drug release

Li Xu, Lidong Shao, Minqi Hu, Lin Chen, and Yunmei Bi

Abstract: A new third-generation thermoresponsive amphiphilic dendron consisting of a hydrophobic poly(benzyl ether) dendritic core and hydrophilic oligo(ethylene glycol) peripheries was synthesized by an efficient convergent approach. Its structure was confirmed by ^{1}H NMR, ^{13}C NMR, IR, GPC, MALDI-TOF MS, and elemental analysis. Turbidity and dynamic light scattering (DLS) measurements demonstrated that the dendron showed a reversible temperature-dependent phase-transition behavior in aqueous solution and its lower critical solution temperature (LCST) was lower than that of the corresponding second-generation dendron, indicating the dependence of LCSTs on the generation of dendrons. Fluorescent spectroscopy and TEM studies revealed that the dendron would self-assemble into nanospherical micelles with a very low critical micelle concentration (CMC) in water. The core-shell structure of the micelles was proved by ^{1}H NMR analyses of the micelles in D_2O. The drug-loading capacity of the dendron micelles is about 29 wt % for podophyllotoxin (POD) used as a model drug, and in vitro release tests showed a desired thermoresponsive drug-release behavior. These results indicate that the dendron is promising as stimuli-responsive material for biomedical applications.

Key words: amphiphilic dendron, poly(benzyl ether) dendritic core, oligo(ethylene glycol) peripheries, thermoresponsive micelles, controlled drug release.

Résumé : Utilisant une approche impliquant une synthèse efficace et convergente, on a réalisé la synthèse d'un nouveau dendron amphiphile thermosensible de troisième génération formé d'un coeur dendritique, un poly(éther benzylique) hydrophobe, et des périphéries hydrophiles à base d'oligo(éthylèneglycol). On en a confirmé la structure résonance magnétique nucléaire du ^{1}H et du ^{13}C, infrarouge, chromatographie en phase gazeuse, spectrométrie de masse en temps de vol avec ionisation par désorption au laser d'une matrice (« MALDI-TOF MS ») et par analyse élémentaire. Des mesures de turbidité et de diffusion de la lumière dynamique (DLD) ont permis de démontrer que, en solution aqueuse, le dendron donne lieu à un comportement impliquant une transition de phase réversible en fonction de la température et que sa température de solution critique la plus basse (TSCB) est plus basse que celle du dendron correspondant de deuxième génération, ce qui met en évidence que la température de solution critique la plus basse (TSCB) varie en fonction de la génération des dendrons. Des études de spectroscopie de fluorescence et de microscopie électronique de transmission (MET) mettent en évidence le fait que, dans l'eau, le dendron pourrait donner un auto-assemblage en micelles nanosphèriques avec une concentration micellaire critique (CMC) très basse. Faisant appel à des analyses de RMN du ^{1}H, on a pu déterminer que, dans le D_2O, la structure des micelles correspond à coeur en forme de coquille. La capacité de charge de médicament par les micelles de dendron est d'environ 29 % en poids pour la podophyllotoxine (POD) utilisée comme médicament modèle et des essais d'émission in vitro ont permis de démontrer un comportement recherché d'émission thermosensible des médicaments. Ces résultats indiquent que le dendron est un matériel stimuli-sensible prometteur pour des applications biomédicales.

Mots-clés : dendron amphiphile, poly(éther benzylique) à coeur dendritique, oligo(éthylèneglycol), périphéries, micelles thermosensibles, émission contrôlée de médicament.

[Traduit par la Rédaction]

Introduction

In recent years, a growing interest has been evident in the development of dendrimers that are responsive to external stimuli, such as pH,[1,2] temperature,[3,4] light,[5,6] and redox state.[7,8] One of the most appealing stimuli-responsive species is the thermoresponsive dendrimer, since such a system combines the advantages of temperature sensitivity and dendritic effect[9] for applications such as biomedicine and catalysis.[10–13] Thermoresponsive dendrimers can be constructed by attaching thermoresponsive groups or polymers to the periphery or the core of the dendrimer. Dendrimers with properly balanced hydrophilic and hydrophobic units can also act as temperature-sensitive dendrimers.[14] Poly(*N*-isopropylacrylamide) (PNIPAM) is a representative polymer exhibiting thermoresponsive behavior. It shows a dissolution–precipitation transition

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in aqueous solution at 32 °C. *N*-Isopropylacrylamide (NIPAM) has been incorporated into linear and dendritic macromolecules to obtain thermoresponsive copolymers and dendrimers.[15–20] For example, Kimura et al.[21] prepared the first thermoresponsive dendrimer by polymerization of NIPAM from the termini of a poly(propyleneimine) dendrimer with terminal thiol groups. The core of the dendrimer was a cobalt complex, which catalyzed oxidation of the thiol compounds.

Polyethylene glycol (PEG), an uncharged, nontoxic, and nonimmunogenic water-soluble polymer, has found diverse applications in biological sciences, personal care products, and industrial additives.[22] However, PEG possesses a lower critical solution temperature (LCST) above 100 °C, which is too high and may be a limitation to its widespread use in the biomedical field.[23] Recently, PEGylated polymers have been explored by incorporating PEG or oligo(ethylene glycol) (OEG) units into other macromolecules. They show lower LCSTs and are promising alternatives to PNIPAM for applications in drug delivery systems.[24–28] PEG is also useful for the preparation of temperature-sensitive dendrimers. For example, Li et al.[29] synthesized dendrimers containing temperature-sensitive oligoethylene glycol as a building block. These unique dendrimers showed fast and sharp transitions around their LCSTs. Their thermoresponsive behaviors are dependent on both the generation and the terminal structure.

Some amphiphilic PEGylated dendrons that are parts of dendrimers have temperature-dependent phase-transition behavior. Moreover, these dendrons, composed of hydrophobic and hydrophilic parts, can self-assemble into dendritic micelles. For instance, Asthmanikandan et al.[9] reported on thermosensitive biaryl-based dendrons with a pentaethylene glycol monomethyl ether and a decyl chain. These amphiphilic dendrons exhibited generation-dependent temperature sensitivity and were capable of forming micelles and reverse micelles in polar and apolar solvents, respectively. Chang and Dai[30] synthesized dendrons consisting of hydrophobic oligo (*p*-phenylene vinylene) core branches and hydrophilic oligo (ethylene oxide) terminal chains. These dendrons showed a LCST and the critical micellization behavior. The unusual properties of these amphiphilic PEGylated dendrons are attractive for potential application in controlled drug delivery systems.

Usually, the purification of synthesized dendrons or dendrimers requires column chromatography, which not only is tedious to perform but also unavoidably leads to losses of product. In this paper, we described the column-free synthesis, and thermoresponsive and micellar behaviors of a third-generation amphiphilic dendron containing a hydrophobic poly(benzyl ether) dendritic core and 27 hydrophilic diethylenoxy methyl ethers at its periphery. The controlled release of the model drug podophyllotoxin (POD) using the thermoresponsive micelles was also studied in detail.

Results and discussion

Synthesis and characterizations of dendrons

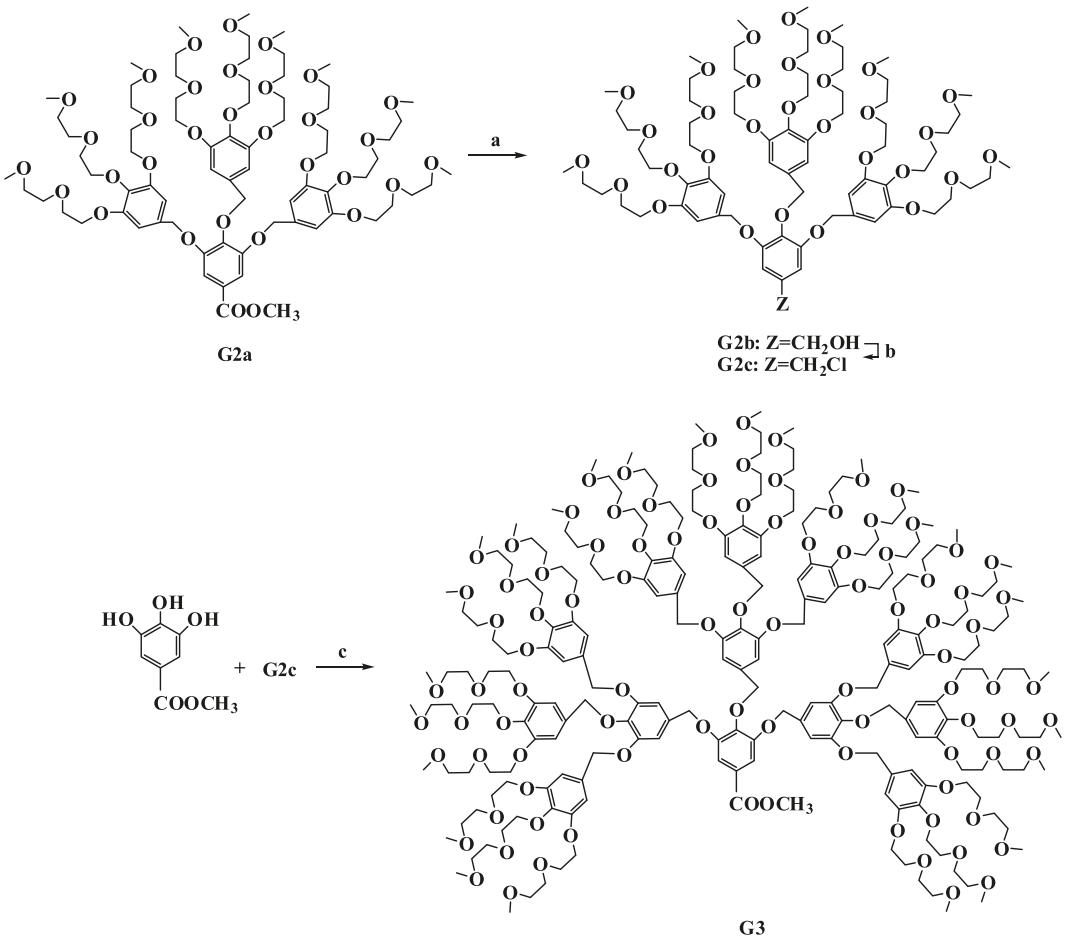
The synthetic procedures of the dendrons are summarized in Scheme 1. The second-generation dendron **G2a** was synthesized according to our previously reported method.[31] **G2a** was reduced with lithium aluminum hydride (LAH) to give the second-generation dendron alcohol **G2b**. Chlorination of **G2b** with $SOCl_2$ afforded the corresponding chloride **G2c**. In this study, each dendron was purified simply by solvent extraction or dialysis to remove the earlier generation dendron. Compared with the synthetic strategy of the corresponding first-generation dendron, the reaction conditions to synthesize **G2b** were modified by adjusting the molar ratio of LAH to **G2a** from 2:1 to 3:1 and prolonging the reaction time from 16 to 20 h. The reaction time of **G2b** with $SOCl_2$ was also prolonged from 3 to 4 h. As a result, the purification of **G2b** and **G2c** required only solvent extraction. The alkylation of methyl gallate with **G2c** by using K_2CO_3 as the base in *N*, *N*-dimethylformamide (DMF) provided analytically pure third-generation dendron **G3**, requiring only a dialysis workup. None of the dendrons required column chromatography for analytically pure material, thus facilitating scale-up. All new compounds were obtained as oily liquids and their structures were characterized by ^{1}H NMR and ^{13}C NMR spectra (Supplementary data Figs. S1–S3; Fig. 1), FT-IR, as well as ESI-MS or MALDI-TOF MS. **G3** was further characterized by GPC and elemental analysis. GPC analysis of **G3** showed a single peak (PDI = 1.05, Fig. 2). The M_n measured by GPC for **G3** was much lower than the calculated one using calibration with linear polystyrene. This is attributed to the difference between the hydrodynamic volumes of polystyrene and **G3** and is consistent with the studies of polyether dendrimers by Fréchet and coworkers.[32] However, MALDI-TOF MS of **G3** showed a single signal at m/z ($[M + Na]^+$) = 4624 (calcd mass, 4599), indicating the formation of **G3**.

Formation of micelles

The presence of a hydrophobic poly(benzyl ether) branch unit and hydrophilic oligo(ethyleneoxy) periphery means that these amphiphilic dendrons can potentially be precursors to nanoparticles with an oligo(ethyleneoxy) corona and a poly (benzyl ether) core upon dissolution in water, which is a selective solvent. The formation of the hydrophobic poly(benzyl ether) domain can be confirmed by ^{1}H NMR spectra with $CDCl_3$ and D_2O as locking solvents for **G3**. In $CDCl_3$, where micellar formation was not expected, all ^{1}H NMR resonances attributed to poly(benzyl ether) and oligo(ethyleneoxy) units were detected as shown in Fig. 1*a*. However, the ^{1}H NMR spectrum in D_2O showed an intensity reduction of poly(benzyl ether) resonances resulting from the suppressed molecular motion of the aggregated hydrophobic branch units (Fig. 1*b*). The small broad signals in the NMR spectrum indicate restricted motions of these protons within the core of nanoparticles. This result is evidence for a core-shell type formation with a hydrophobic inner core and a hydrophilic shell. Similar trends in ^{1}H NMR spectra are consistent with amphiphilic block copolymer systems.[33,34]

The formation of micelles from the third-generation dendron **G3** was also examined by the detection of critical micelle concentration (CMC) relying on a fluorescence technique with pyrene as the probe because pyrene preferentially partitions into the hydrophobic core of micelles from water.[35] The intensity ratio of I_{341}/I_{337} vs the logarithm of concentrations of **G3** in the pyrene excitation spectra was plotted in Fig. 3*a*. It is seen that the I_{341}/I_{337} ratios remain fairly constant below a certain concentration and then increase

Scheme 1. Synthesis of dendron **G3**. Reagents and conditions: (*a*) $LiAlH_4$ (LAH), tetrahydrofuran (THF), room temperature (rt), 20 h; (*b*) $SOCl_2$, *N*, *N*-dimethylformamide (DMF), dichloromethane (DCM), rt, 3 h; (*c*) K_2CO_3, DMF, 70 °C, 24 h.



remarkably above that concentration, indicating the formation of micelles. From this plot, a CMC of 7.7×10^{-6} mol/L was obtained from the intersection of two straight lines: the baseline and the rapidly rising I_{341}/I_{337} line (Fig. 3*b*), which is lower than the one of the second-generation dendron **G2a** (2.2×10^{-5} mol/L).[31] This could be attributed to the fact that **G3** is larger than **G2a**, which facilitates the aggregation of the dendron. These CMC values are comparable to those reported for some other amphiphilic dendrons and linear copolymers.[9,36] The low CMC values mean that the dendrons can self-assemble to form nanoscaled core-shell micelles at a lower compound concentration, which is suitable for drug delivery in very dilute aqueous milieux such as body fluids.[37]

Micelle morphology was investigated by transmission electron microscopy (TEM). It can be seen from Fig. 4 that dendron **G3** forms spherical micelles with a core-shell (corona) structure and an average diameter of about 60 nm. The micelles made from **G3** were bigger than those from **G2a**[31] because of its larger core and peripheries.

Thermoresponsive behavior

To investigate whether the third-generation dendron **G3** exhibits a thermal response, we examined the optical transmittance of a **G3** aqueous solution as a function of temperature. The concentration dependence of the LCST was also investigated in the range of 0.1–2.1 mg/mL and the results are plotted in Fig. 5. For comparison, the respective curve of dendron **G2a**[31] was also added (Fig. 5*b*). Dendron **G3** displayed an obvious phase transition in water and its LCST values decreased with increasing concentration from 0.3 to 1.5 mg/mL. Compared with dendron **G2a**, **G3** shows lower LCSTs at all concentrations tested, demonstrating the dependence of LCSTs on the generation of dendrons, which was in correspondence with the report by Aathimanikandan et al.[9] The reason may arise from the increased size of the dendrons with the increasing generation; thus, the interaction of the peripheral oligo(ethyleneoxy) groups would take place more efficiently with the increasing generation of the dendron, which leads to the dehydration of the terminal oligo (ethyleneoxy) groups at a lower temperature with increasing generation.[3]

The thermoresponsive behavior of dendrons **G2a** and **G3** was also analyzed by dynamic light scattering (DLS). The hydrodynamic diameter (D_h) of the dendrons are apparently influenced by the temperature, as shown in Fig. 6. At temperatures lower than the LCSTs, the two dendrons are easily solubilized in water so that the amphiphilic dendrons self-assemble into core-shell micelles with a low D_h of approximately 50–60 nm. However, a dramatic increase in D_h was

Fig. 1. ^{1}H NMR spectra of **G3** in $CDCl_3$ (*a*) and D_2O (*b*).

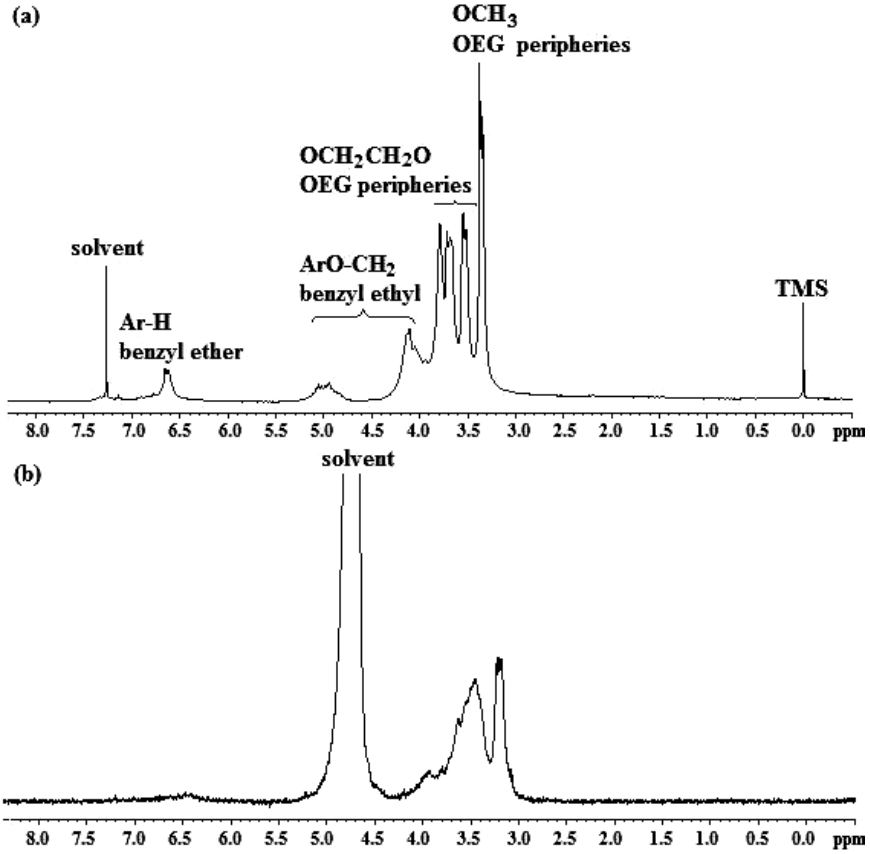
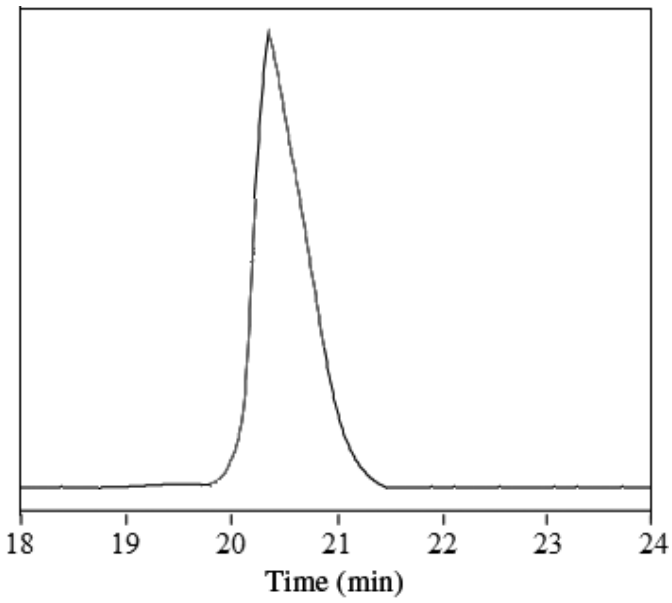


Fig. 2. GPC trace of **G3** with M_n (theory) = 4599 Da and M_n (GPC) = 1666.



observed once the temperature increased to near the LCST (58 and 54 °C, respectively), which indicated dendron aggregation. Dendron **G2a** requires a higher temperature to induce aggregation than dendron **G3** does. The aggregation process was completely reversible, and the aggregates became soluble again below the onset temperature. These are consistent with the results of turbidimetry measurement.

Controlled drug release

Hydrophobic drugs can be physically incorporated and stabilized in the micellar hydrophobic inner core by hydrophobic interaction. Podophyllotoxin (POD), an anticancer drug with poor solubility in water, was used as a model drug to investigate the potential application of dendron **G2a** and **G3** micelles in controlled drug release. The amount of drug incorporated into the micelles was measured by a UV spectrometer. The results showed that POD was successfully incorporated into micelles of **G2a** and **G3**, and the drug-loading content (DLC) and entrapment efficiency (EE) were about 21.24% and 77.81% for **G2a**, and 28.71% and 94.95% for **G3**, respectively, which are higher than those of the traditional micelles.[37] Furthermore, the amount of POD introduced into the micelle was dependent on the generation of the dendrons. The DLCs and EEs of **G3** with more hydrophobic phenyl groups are higher than those of **G2a** with fewer hydrophobic phenyl groups. To obtain information about the release of POD from **G2a** and **G3** micelles, the drug-loaded **G2a** and **G3** micelles were dissolved in PBS and dialyzed against PBS. Figure 7 shows the release profile of POD from **G2a** and **G3** micelles. In contrast with the rapid release of the free drug from a cellulose membrane (99% released in 4 h), the release from the dendritic micelles was slow and showed sustained characteristics. The generation of dendrons also affects drug release. The amount of drug released and the rate of release were greater for **G3** than for **G2a** micelles. This could be ascribed to steric hindrance of crowded end functional groups in higher generations and weaker interaction between these functional groups

Fig. 3. (*a*) Excitation spectra of pyrene in water at different concentrations of **G3**. (*b*) Plot of the intensity ratio I_{341}/I_{337} vs the logarithm of the concentration of **G3**.

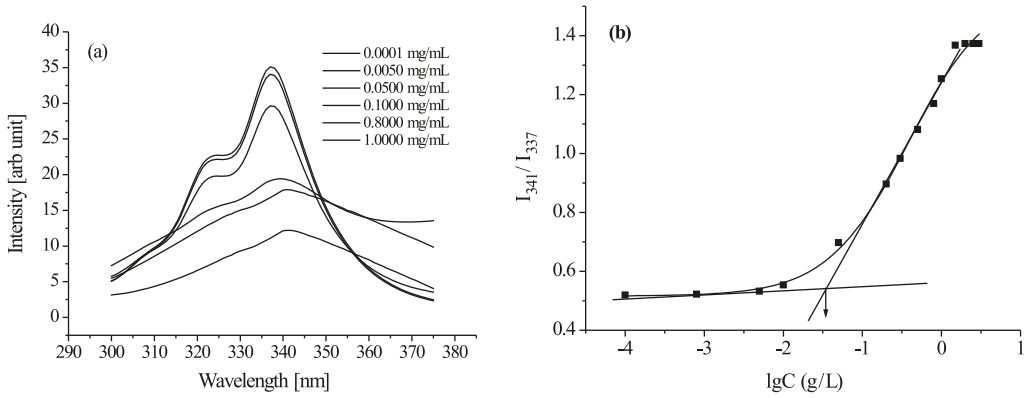
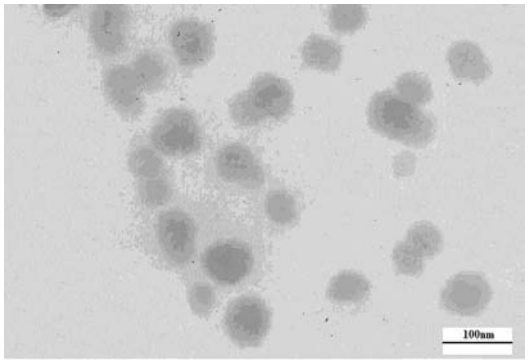


Fig. 4. Transmission electron microscopy (TEM) image of **G3** micelles.



and the drug molecules. A similar trend was observed in linear-dendrimer block copolymers containing PEG studied by Adeli et al.[38] To evaluate the effect of the temperature stimulus on release of POD from the thermoresponsive micelles, the POD release from **G3** micelles was examined in PBS solution at 25, 37, and 55 °C. As shown in Fig. 8, the POD release rate and amount increased with increasing temperature under constant pH (7.4) . A slow release was observed owing to the greater stability of micelles when the temperature was below the LCST (20 and 37 °C). However, when the temperature was above the LCST (55 °C), drug release was accelerated because of the temperature-induced structure change of the micelles. At a temperature above the LCST, the hydrophilicity of the micellar shell, comprised of oligo(ethyleneoxy) chains, was weakened, resulting in the deformation of the structure of the micelles. Thus, the drug release was faster above the LCST than below the LCST. Unfortunately, there was little change in the release rate of POD from **G3** micelles as a function of temperature, probably because the drug would be retained in the hydrophobic aggregates above the LCST. Therefore, this may not be an ideal system for thermally triggered drug release. However, it is expected that a less hydrophobic drug may be more readily released from **G3** micelles above the LCST.

Conclusions

We synthesized a novel third-generation thermoresponsive amphiphilic dendron with a hydrophobic poly(benzyl ether) dendritic core and hydrophilic oligo(ethylene glycol) peripheries using a simple convergent approach. The dendron exhibited a thermally induced phase transition in aqueous solution and its LCST increased with decreasing concentration over the range of 0.3–2.1 mg/mL of the dendron in aqueous solution. Studies of the association behavior of the synthesized dendron in aqueous solution revealed that the dendron is capable of forming spherical micelles of around 60 nm in diameter. Compared with its second-generation counterpart, the dendron exhibited lower LCST and CMC. The self-assembled dendron micelles exhibited high drug-loading capacity and thermoresponsive drug release behavior. These results indicate that the thermosensitive amphiphilic dendron possesses promising potential applications in the biomedical field.

Experimental

Instruments and materials

^{1}H NMR and ^{13}C NMR spectra were determined on a Bruker DRX-500 with TMS as internal standard. ESI-MS spectra were obtained on a Finnigan LCQ-Advantage mass spectrometer. Matrix-assisted laser desorption ionization (time of flight) mass spectrometry (MALDI-TOF MS) was carried out on a Bruker Biflex III MALDI-TOF spectrometer with α-cyano-4-hydroxylcinnamic acid (CCA) as the matrix. The GPC system consisted of a Waters 2690D separations module and a Waters 2414 refractive index detector (RI). Styragel HR1 THF and HR2 THF columns (Waters) were used at 40 °C with polystyrene as the standard and tetrahydrofuran (THF) as the mobile phase at a flow rate of 0.3 mL/min. FT-IR spectra were taken on a Nicolet AVATAR 360 FT-IR spectrometer.

Methyl 3,4,5-tris(3,4,5-tris(2-(2-methoxyethoxy)ethoxy) benzyloxy) benzoate (**G2a**) was synthesized according to the method previously reported.[31] Methyl gallate was purified by recrystallization from methanol. THF was refluxed on sodium and distilled from sodium benzophenone. DMF and dichloromethane (DCM) were distilled from anhydrous calcium chloride for drying. Other reagents were used as received.

Synthesis of 3,4,5-tris(3,4,5-tris(2-(2-methoxyethoxy) ethoxy)benzyloxy) benzyl alcohol (G2b)

Dendron **G2b** was prepared by the reduction of **G2a** with

Fig. 5. (*a*) Transmittance measurements as a function of temperature for different concentrations of **G3**. (*b*) Dependence of lower critical solution temperatures (LSCTs) on concentrations of **G3** and **G2a.**

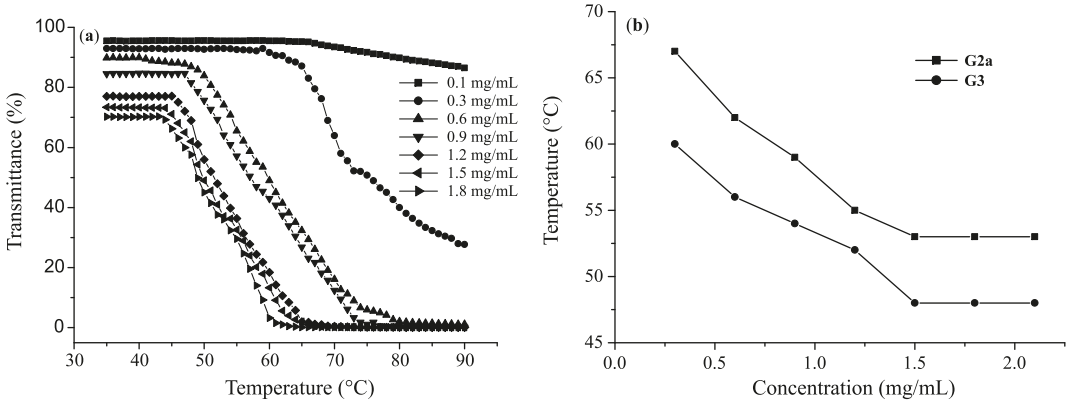


Fig. 6. Plots of hydrodynamic diameters (D_h) of the micelles from **G3** and **G2a** in aqueous solution as a function of temperature from dynamic light-scattering (DLS) measurements.

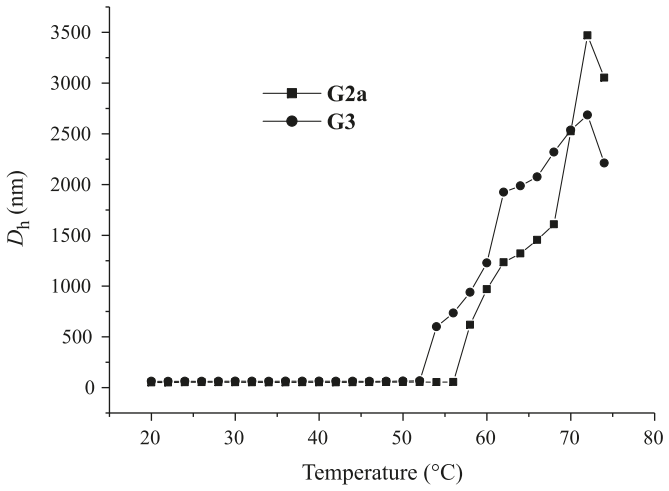
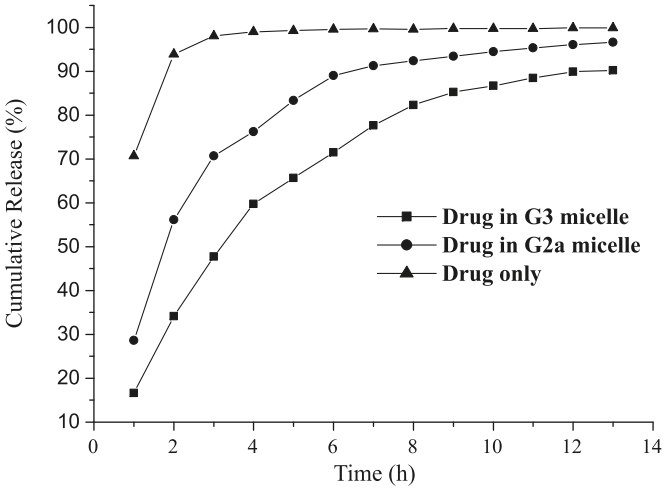
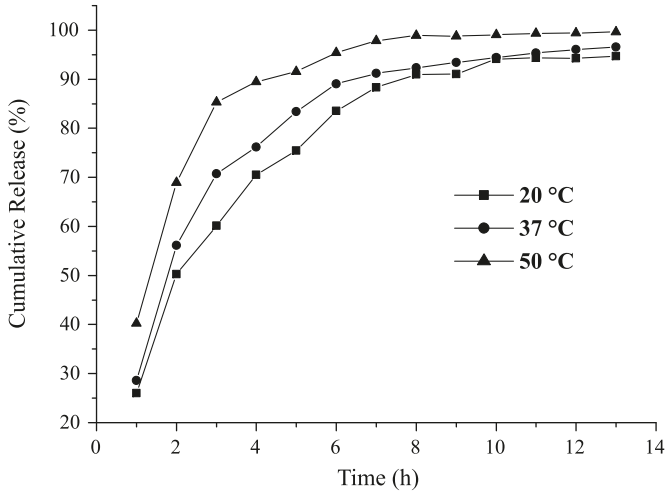


Fig. 7. In vitro release profile of podophyllotoxin from **G3** and **G2a** micelles at 37 °C.



LAH. To a solution of **G2a** (2.43 g, 1.6 mmol) in dry THF was added LAH (0.20 g, 5.2 mmol) at –5 °C. This mixture was stirred for 0.5 h, and then warmed to room temperature.

Fig. 8. Temperature-responsive drug release from **G3** micelles in phosphate-buffered saline solutions (PBS, pH 7.4) at 20, 37, and 55 °C, respectively.



After 20 h, the reaction was quenched by dropwise addition of 10% NaOH. The resulting precipitate was filtered and the THF was evaporated. The residue was dissolved in DCM and washed with saturated NaCl solution. The organic phase was dried over $MgSO_4$. After filtration, the DCM was rotoevaporated, affording **G2b** as a pale yellow oil, yield 80.5%. IR (KBr) *ν*: 3445 (m, ν(OH)), 2929 (s), 2879 (s), 1591 (s, ν(Ar)), 1501 (m, ν(Ar)), 1112 (s, ν_{as}(COC)). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.64 (m, 8H, Ar H), 4.96–5.00 (m, 6H, CH_2), 4.53 (s, 2H, CH_2), 3.95–4.18 (m, 18H, CH_2), 3.77–3.85 (m, 18H, CH_2), 3.68–3.72 (m, 18H, CH_2), 3.53–3.55 (m, 18H, CH_2), 3.36–3.38 (m, 27H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 152.95, 152.68, 138.19, 137.88, 133.74, 133.02, 108.23, 107.51, 107.02, 75.09, 72.65, 72.28, 72.22, 71.53, 70.84, 70.60, 69.95, 69.11, 64.73, 59.15. ESI-MS *m/z*: 1513 $[M + Na]^+$.

Synthesis of 3,4,5-tris(3,4,5-tris(2-(2-methoxyethoxy) ethoxy)benzyloxy) benzyl chloride (G2c)

G2c was obtained by chlorination of **G2b** with $SOCl_2$. **G2b** (3.88 g, 2.6 mmol) was dissolved in 50 mL of dry DCM. DMF (4.5 mL) and $SOCl_2$ (0.93 g, 7.8 mmol) was then added dropwise and the reaction mixture was stirred for

another 3 h. After being washed with aqueous $NaHCO_3$ solution and saturated NaCl, the organic phase was dried over $MgSO_4$ and filtered, and the solvent was rotoevaporated, affording **G2c** as a pale yellow oil, yield 92.4%. IR (KBr) *ν:* 2929 (s), 2879 (s), 1589 (s, ν(Ar)), 1499 (m, ν(Ar)), 1112 (s, ν_{as}(COC)). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.63–6.65 (m, 8H, Ar H), 5.00 (s, 6H, CH_2), 4.46–4.49 (m, 2H, CH_2), 3.98–4.19 (m, 18H, CH_2), 3.77–3.87 (m, 18H, CH_2), 3.65–3.72 (m, 18H, CH_2), 3.51–3.64 (m, 18H, CH_2), 3.34–3.38 (m, 27H, CH_3). ESI-MS *m/z*: 1531 $[M + Na]^+$.

Synthesis of methyl 3,4,5-tris(3,4,5-tris(3,4,5-tris(2-(2-methoxyethoxy)ethoxy)benzyloxy)benzyloxy)benzoate (G3)

G3 was obtained by the alkylation of methyl gallate with **G2c**. A mixture of methyl gallate (0.09 g, 0.5 mmol), **G2c** (2.41 g, 1.6 mmol), and K_2CO_3 (0.69 g, 5.0 mmol) in dry DMF was stirred and heated to 70 °C for 24 h under a nitrogen atmosphere. After filtration and then evaporation of DMF in vacuo, the residue was dissolved in a small amount of distilled water, which was dialyzed against distilled water for 3 days. The water in the dialyzed solution was removed under reduced pressure, affording **G3** as a pale yellow oil, yield 62.3%. IR (KBr) *ν*: 2937 (m), 2878 (m), 1723 (m, ν(C=O)), 1589 (m), 1503 (m), 1110 (s, ν_{as}(COC)) ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.61–6.66 (m, 26H, Ar H), 4.94–5.07 (m, 24H, CH_2), 3.92–4.13 (m, 54H, CH_2), 3.78–3.79 (m, 57H, CH_2 + CH_3), 3.68–3.71 (m, 54H, CH_2), 3.52–3.55 (m, 54H, CH_2), 3.35–3.38 (m, 81H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 166.72 (C=O), 154.66, 153.64, 153.02, 152.79, 151.85, 149.46, 142.49, 141.52, 138.31, 136.42, 133.74, 133.02, 132.71, 131.85, 131.65, 110.19, 109.60, 107.46, 107.05, 75.86, 75.33, 73.27, 72.89, 72.70, 72.34, 72.26, 70.87, 70.66, 69.99, 69.15, 60.46, 59.20, 58.29, 52.50. MALDI-TOF MS *m/z* calcd for $C_{227}H_{350}O_{95}$: 4599; found: 4624 $[M + Na]^+$. Anal. calcd for $C_{227}H_{350}O_{95}$: C 59.28, H 7.67; found: C 59.01, H 7.81.

Fluorescence measurements

Fluorescence spectra were obtained on a Hitachi F-4500 luminescence spectrophotometer using pyrene as a fluorescent probe. Aliquots of pyrene solution (5.9×10^{-6} mg/mL in acetone, 50 μL) were added to volumetric flasks and the acetone was evaporated overnight. Then, aqueous solutions of **G3** at different concentrations were added to the flasks. The final pyrene concentration in each sample was 5.9×10^{-7} mg/mL. The solutions were kept at room temperature for 24 h to equilibrate the pyrene and the micelles. The emission spectra were recorded from 250 to 500 nm with an excitation wavelength of 390 nm.

TEM measurements

A drop of aqueous dendron solution (1 mg/mL) was placed on a Formwar-coated copper grid and dried in air. TEM observation of the micelles was conducted on a Hitachi H-600 instrument operating at an acceleration voltage of 80 kV.

LCST measurement

The LCST was determined by cloud point measurement. Dendron **G3** was dissolved in deionized water at different concentrations. The temperature of the solutions was raised at a constant rate (0.4 °C/min) and the optical transmittance of the polymer solution was measured at 500 nm with a UV–vis spectrophotometer (Cary 50, Varian). The LCST was defined as the temperature corresponding to the initial break points in the resulting transmittance vs temperature curve.[39]

Dynamic light-scattering (DLS) experiments

The D_h of the micelles was determined by DLS as a function of temperature. The measurements were performed with a Zetasizer ZEN 3600 instrument (Malvern, UK) using a light-scattering apparatus equipped with a He–Ne laser. The scattering angle was kept at 173° (backscattering), and the wavelength in the vacuum was set as 633 nm during the whole experiment. Solutions of dendrons **G3** and **G2a** in deionized water (1.0 mg/mL) were filtered with a 0.45 μm filter prior to use. Three replicates were performed for each measurement and an average value was obtained.

Drug loading and in vitro release

Dendron **G2a** or **G3** (50 mg) and POD (15 mg) were dissolved in 10 mL of acetone. Deionized water (10 mL) was added dropwise to the solution under vigorous stirring. The solution was stirred in an open air system overnight to remove acetone by evaporation. The precipitate of unbound POD was removed by centrifugation at 4000 rpm for 30 min. The amount of POD entrapped in the dendron micelles was determined by measuring the absorbance at 292 nm, using a standard calibration curve experimentally obtained with POD–ethanol solutions. The DLCand EE were calculated from the following equations:[37] DLC (wt %) = 100(mass of POD in micelles / mass of POD-loaded micelles); EE (%) = 100(mass of POD in micelles / mass of POD initially loaded).

To study in vitro drug release, a dialysis bag (molecular weight cut off = 3500 Da) containing 15 mL of the drug-loaded micelles in suspension was immersed in a phosphate buffer solution (150 mL, 0.01 mol/L, pH 7.4) at 20, 37, or 55 °C. At predetermined time intervals, 2 mL aliquots of the aqueous solution were withdrawn from the release media for UV–vis analysis, and the same volume of a fresh buffer solution was added. The amount of drug released from micelles at different temperatures was measured by UV absorbance at 292 nm.

Supplementary data

Supplementary data are available with the article through the journal Web site http://nrcresearchpress.com/doi/suppl/10.1139/v2012-038.

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