

Synthesis and Characterization of a Poly(amide amine) Dendrimer Disulfide Having Hydroxy Groups as the Terminals

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(Received November 9, 2001)

A Poly(amide amine) dendrimer with a diphenyl disulfide at the core was synthesized and well characterized, and then used for a host molecule for an organic dye. The molecule shows a reversible redox behavior between the dendron and the dendrimer, being achieved under chemical reactions. Furthermore, a marked difference of encapsulating fashion was observed as the structure changed from dendrimer to dendron.

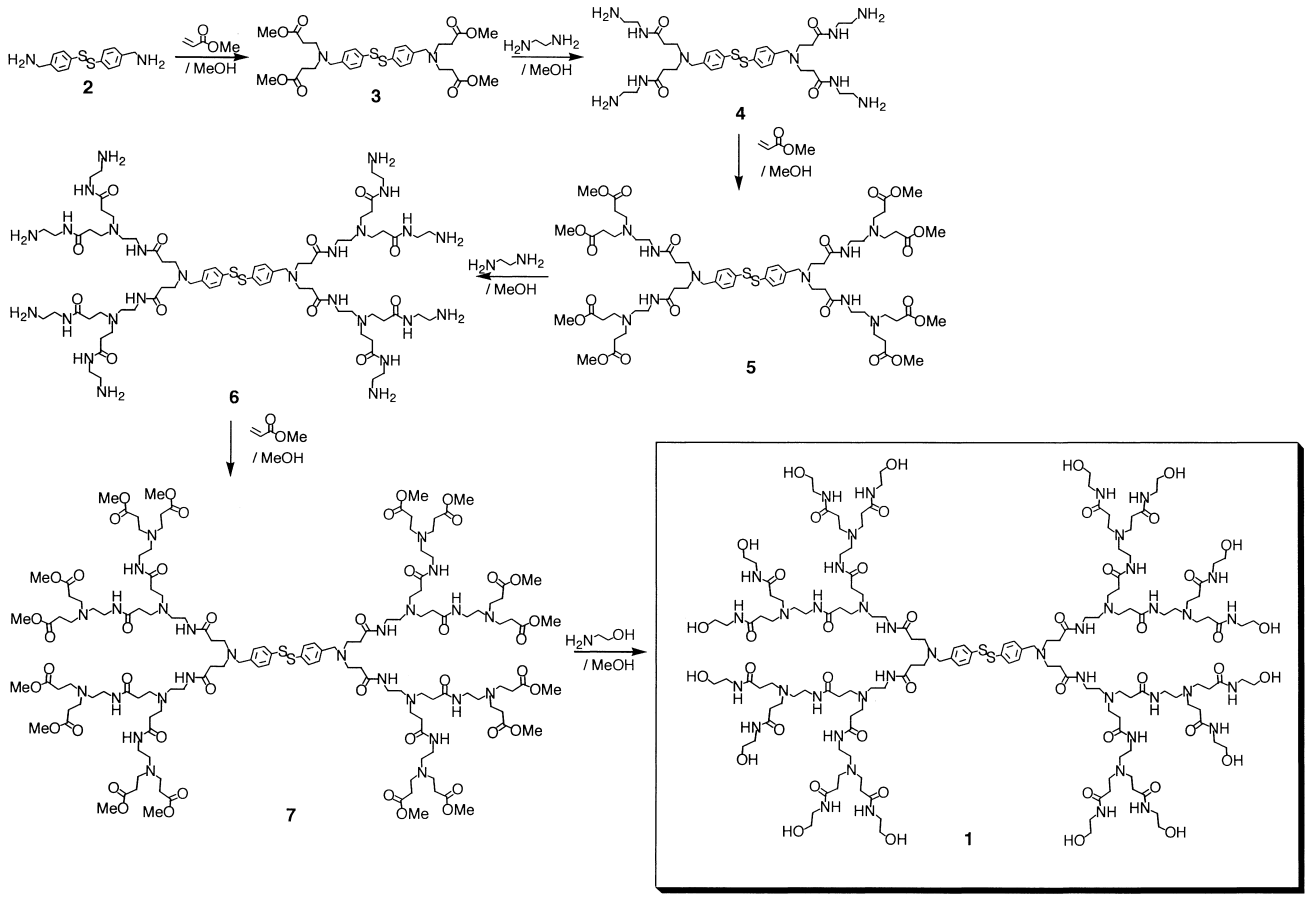
Dendritic macromolecules (dendrimers) have received considerable attention during the past two decades.[1] Since the possibility of encapsulating guest molecules in a dendritic host was proposed in 1982,[2] guest–host systems made out of dendrimers have been one of the main applications of dendritic macromolecules.[1c,3–7] Several host–guest systems employing poly(amide amine) (PAMAM) dendrimers, such as penetration of asperin,[4] and the extraction of a hydrophilic guest (e. g., copper(II) ion[5] and methyl orange[6]) into nonpolar solvents from aqueous phases, have already been developed. Meanwhile, in practical applications (e. g., drug delivery), reversible configurational and constitutional changes of a dendritic host should play an important role. For this reason, there is an increasing focus on developing applications of dendrimers that respond to environmental stimuli.[7,8] Dendrimers having a stimuli-responsive switch by using an intramolecular process (e. g., isomerization of azobenzene unit[7,9]) have been extensively reported. However, much less is known about the structural control between a dendrimer and a dendron via an intermolecular reaction. Recently, we reported that dendrons having a stimuli–responsive switch at the focal point underwent reversible bond formation to give dendrimers.[10] Because of these enormous structural changes via intermolecular reactions, it is expected that a marked difference occurs to a host-guest system of the molecule. This paper describes the synthesis, characterization, and controlling morphology of a poly(amide amine) dendrimer (**1**) having a disulfide bond at the core and hydroxy groups as the terminals, although a dendrimer having interior switchable moieties is quite rare. Furthermore, a marked difference in the encapsulating fashion with the structural control between the dendrimer and the dendron has been reported.

Results and Discussion

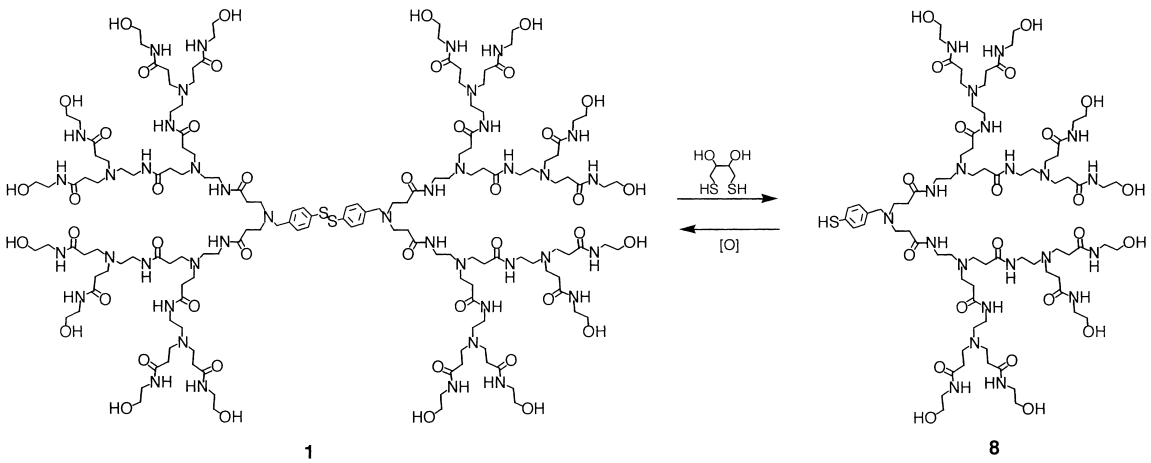
Poly(amide amine) dendrimer disulfide (**1**) was prepared by using a methodology developed by Tomalia and co-workers (Scheme 1).[11] The treatment of a bis(aminomethylphenyl) disulfide (**2**) (G = 0.0), a core of dendrimer, with methyl acrylate produced a dendrimer (**3**) (G = 0.5) in 57% yield. Subsequently, **3** was allowed to react with ethylenediamine to give a dendrimer (**4**) (G = 1.0) in 83% yield. This two-step process could be repeated to prepare dendrimers (**5**) (G = 1.5), (**6**) (G = 2.0), and (**7**) (G = 2.5) in 57, 84, and 69% yields. A surface group transformation of dendrimer **7** was easily accomplished by reacting the PAMAM dendrimer's terminal ester groups with 2-aminoethanol in a methanol solution to afford dendrimer **1** in 56% yield. The structure of **1** was confirmed by ^{1}H, ^{13}C NMR spectroscopies, and MALDI-TOF MS. The MALDI-TOF spectrum of dendrimer **1** clearly showed the parent peak at *m*/*z* 3489.67, which is consistent with the molecular weight of the dendrimer **1** ($[M+H]^+$, calcd 3489.26), as shown in Fig. 1 (a).

It is well-known that disulfide bonds undergo a bond cleavage upon reduction. In order to clarify the utility of the disulfide group as a chemo-switch, we examined the reduction of **1** with dithiothreitol to a dendron thiol (**8**). A methanol solution of the dendrimer **1** was allowed to react with dithiothreitol at room temperature under a nitrogen atmosphere for 1 h to give dendron **8** in 100% yield (Scheme 2). The structure of **8** was confirmed by ^{1}H, ^{13}C NMR spectroscopies, and MALDI-TOF MS. Two doublet signals at 7.31 and 7.46 ppm, which were assigned to the aromatic protons at the core of dendrimer **1** disappeared, and new doublet peaks appeared at δ 6.89 and 7.19, which is consistent with the thiol structure. The MALDI-TOF spectrum of dendrimer **8** clearly showed the parent peak at *m*/*z* 1746.14, which is consistent with the molecular weight of the dendron **8** ($[M + H]^+$, calcd 1745.83), as shown in Fig. 1 (b). Furthermore, dendron **8** reverted back to dendrimer **1** upon a treatment with oxygen bubbling at room temperature for 1 h in 100% yield (Scheme 2). These results indicated that dendrimer **1** is reversibly switched to dendron **8**.

In order to clarify the utility of the dendrimer disulfide **1** as a molecular capsule, the host–guest properties of **1** were investigated in buffered aqueous media at pH 7 using Phenol Blue (PB) as guest molecules. Brooker and Sprauge suggested the use of PB as a solvent property indicator; the absorption maxi-



Scheme 1. Synthesis of dendrimer disulfide **1**.



Scheme 2. Structural control of dendrimer **1**.

mum (668 nm in water, 658 nm according to our measurement at 25 °C) shifts to shorter wavelengths in nonpolar solvents (e. g., 552 nm in cyclohexane).[12] The blue shift is attributed to destabilization of the polar excited state $PB^{\pm}$. Some of the features of the host-guest system of the dendrimer were illustrated with PB by Newkome et al.[3k] Because dendron **8** is unstable and is easily oxidized to give the corresponding dendrimer **1**, we used dendron (**9**) (Chart 1), of which the thiol group was protected by benzylation, to compare the encapsulation properties against that of **1**.[13] The absorption maxima of PB were measured in H_2O at 25 °C as a function of the dendrimer concentration, as summarized in Fig. 2. An examination of Fig. 2, employing dendron **9** as a host molecure, reveals two noteworthy characteristics: (i) the maximum absorption wavelength (λ_{max}) in the presence of **9** shifts to shorter wavelengths (638 nm), though it is not changed in a very dilute solution, and (ii) an aggregate was formed above a concentration which corresponds to the critical micelle concentration (CMC). Hence,

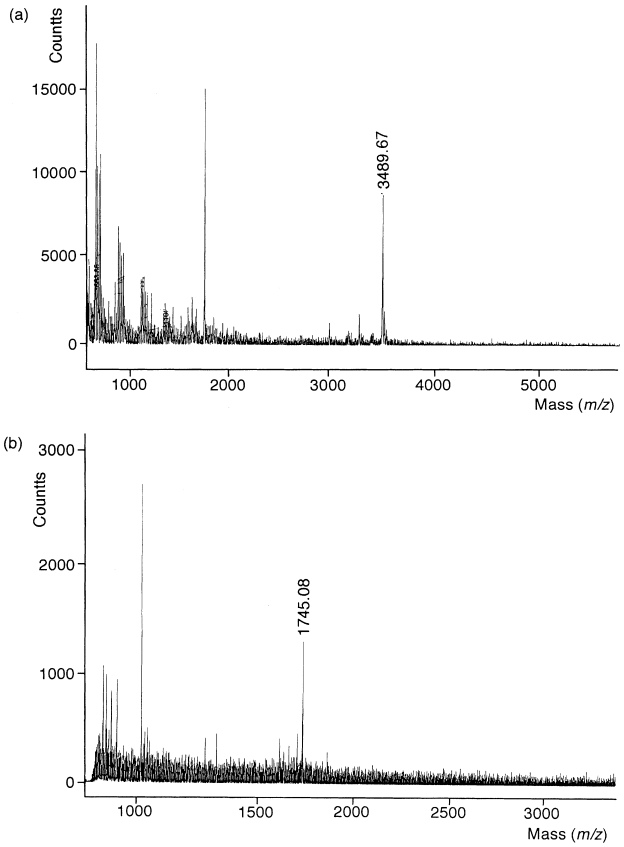


Fig. 1. MALDI-TOF spectra of (a) dendrimer disulfide **1** and (b) dendron thiol **8**.

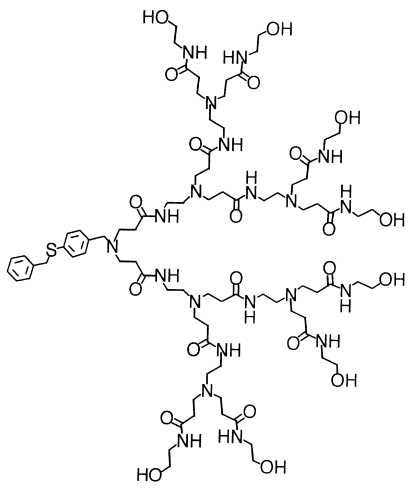


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Chart 1. Structural fomula of **9**.

PB might be captured by the hydrophobic cavity of the aggregate made of dendrons **9**. On the other hand, the red shift (by 52 nm) observed for dendrimer **1** implies that molecule **1** can provide a cavity that is more polar than water. This marked red shift could be elucidated by bifunctional stabilization; i. e. the polar excited $PB^{\pm}$ in the cavity is stabilized both by hydrogen bonding with the amide groups and by an electrostatic interaction with the amine groups of the building block of PAMAM

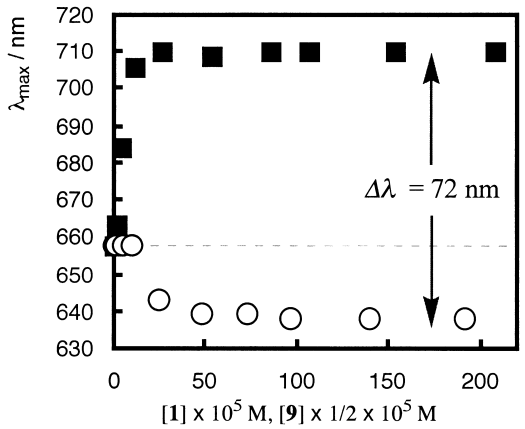

Fig. 2. Absorption maximum of PB (1.0×10^{-5} M) at 25 °C in the presence of [**1**] $\times$ 10^5 M (■), [**9**] $\times$ 1/2 $\times$ 10^5 M (○).

dendrimer.[14] An analogous red shift was observed for the carix[6]arene derivative by Shinkai et al.[15] It is notable that the structure of the dendritic host, dendrimer or dendron, plays a crusial role in the encapsulating behavior and the shift in λ_{max} of PB.

CMC of **9** was also observed and estimated at 0.26 mM by dynamic light scattering (DLS; 25 °C, He–Ne laser). The particle size of the aggregates was 77 $\pm$ 12 nm (3.00 mM). Furthermore, an aggregate made of dendron thiol **8**, of which the particle size was estimated at 55 $\pm$ 5 nm (3.00 mM), was observed by DLS.

The results described herein show the first example of a controlling morphology of a PAMAM dendrimer having a disulfide bond at the core. In addition to a reversible switch between a dendron and a dendrimer being quite rare, a marked difference in the encapsulating fashion was observed as the structure changed from dendrimer to dendron. Further work is in progress to explore the applications and advantages of dendrimer disulfides.

Experimental

NMR spectra were measured on a Bruker AVANCE400 spectrometer. Matrix-assisted laser desorption ionization time-of-flight mass spectroscopy (MALDI-TOF-MS) was performed on a Voyager Elite mass spectrometer using dithranol (1, 8, 9-anthracenetriol) as a matrix. Elemental analysis was carried out on a Perkin-Elmer 2400 CHN elemental analyzer. GPC experiments were performed on a Japan Analytical Industry Co. model LC-918V with GS-320 columns, and methanol was used as the eluting solvent. UV/vis spectra (λ_{max} in nm (ε)) were measured on a Shimazu UV-160A spectrophotometer. Dynamic light scattering (DLS) experiments were performed using Otsuka Denshi DLS-7000.

The reagents were obtained from Wako Pure Chemical Industries Ltd., Tokyo Kasei Co. Ltd., or Aldrich Chemical Co. The reagents used as reaction solvents were further purified by general methods.

Preparation of the Dendrimer Disulfide 3. A mixture of **2** (222 mg, 0.803 mmol), methyl acrylate (3.00 mL, 32.8 mmol), and methanol (4 mL) was stirred at 40 °C for 2 days. After the usual work-up, the residue was purified by silica-gel column chro-

matography (eluent, chloroform) and GPC to afford dendrimer **3** (281 mg, 0.452 mmol) as a thick oil in 57% yield: ^{1}H NMR ($CDCl_3$) δ 2.46 (t, J = 7.20 Hz, 8H), 2.78 (t, J = 7.20 Hz, 8H), 3.55 (s, 4H), 3.63 (s, 12H), 7.21 (d, J = 8.40 Hz, 4H), 7.43 (d, J = 8.40 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 32.5, 49.1, 51.5, 57.8, 127.7, 129.4, 135.6, 138.5, 172.8. Anal. Calcd for $C_{30}H_{40}N_2O_8S_2$: C, 58.04; H, 6.49; N, 4.51%. Found: C, 57.70; H, 6.59; N, 4.41%.

Preparation of the Dendrimer Disulfide 5. A solution of **3** (650 mg, 1.047 mmol) in methanol (30 mL) was added dropwise to ethylenediamine (28.3 mL, 0.419 mol). The mixture was stirred a room temperature for 12 h. After the usual work-up, the residure was reprecipitated from a methanol-ether solution to obtain dendrimer **4**, which was used for following reaciton without further purification.

A mixture of **4** (926 mg, 1.263 mmol), methyl acrylate (18.68 mL, 0.202 mol), and methanol (20 mL) was stirred at 40 °C for 5 days. After the usual work-up, the residue was purified by silica-gel column chromatography (eluent, chloroform/methanol = 30/1) and GPC to afford the dendrimer **5** (1.026 g, 0.722 mmol) as a thick oil in 57% yield: ^{1}H NMR ($CDCl_3$) δ 2.36 – 2.44 (m, 24H), 2.51 (t, J = 5.30 Hz, 8H), 2.74 (t, J = 6.80 Hz, 16H), 2.79 (t, J = 6.80 Hz, 8H), 3.27 (q, J = 5.30 Hz, 8H), 3.60 (s, 4H), 3.65 (s, 24H), 7.04 (t, J = 5.30 Hz, 4H), 7.24 (d, J = 8.20 Hz, 4H), 7.41 (d, J = 8.20 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 33.1, 33.8, 37.5, 49.6, 50.0, 52.0, 53.3, 57.6, 127.9, 130.1, 136.1, 138.0, 172.3, 173.4. Anal. Calcd for $C_{66}H_{104}N_{10}O_{20}S_2$: C, 55.76; H, 7.87; N, 9.85%. Found: C, 55.70; H, 7.69; N, 9.91%.

Preparation of the Dendrimer Disulfide 7. A solution of **5** (472 mg, 0.332 mmol) in methanol (30 mL) was added dropwise to ethylenediamine (32.3 mL, 0.478 mol). The mixture was stirred at room temperature for 2 days. After the usual work-up, the residue was reprecipitated from a methanol-ether solution to obtain dendrimer **6**, which was used for a following reaction without further purification.

A mixture of **6** (456 mg, 0.277 mmol), methyl acrylate (0.51 mL, 5.54 mmol), and methanol (30 mL) was stirred at 40 °C for 5 days. After the usual work-up, the residue was purified by silica-gel column chromatography (eluent, chloroform/methanol = 10/1) and GPC to afford the dendrimer **7** (580 mg, 0.192 mmol) as a thick oil in 69% yield: ^{1}H NMR ($CDCl_3$) δ 2.35 – 2.60 (m, 72H), 2.68 – 3.14 (m, 64H), 3.22 – 3.40 (m, 24H), 3.66 (s, 52H), 7.12 (brs, 8H), 7.26 (d, J = 8.00 Hz, 4H), 7.42 (d, J = 8.00 Hz, 4H), 7.87 (brs, 4H); ^{13}C NMR (CD_3OD) δ 32.4, 33.5, 36.9, 49.0, 49.3, 49.6, 49.8, 51.3, 52.2, 52.6, 57.5, 127.2, 129.4, 135.2, 137.9, 172.2, 172.2, 172.8; MALDI-TOF MS for $C_{138}H_{233}N_{13}O_{44}S_2$: m/z calcd, 3024.65[MH^+]; Found: 3023.19.

Preparation of the Dendrimer Disulfide 1. To a solution of dendrimer disulfide **7** (0.40 g, 0.132 mmol) in methanol (5 mL) was added 2-aminoethanol (19.0 mL, 317 mmol). The mixture was stirred for 5 days at room temperature. After the usual work-up, the residue was purified by GPC to afford the dendrimer disulfide (**1**) (0.26 g, 0.074 mmol) as a light-yellow thick oil in 56% yeild: ^{1}H NMR (400 MHz, CD_3OD) δ 2.37 – 3.75 (m, 244H), 7.31 (d, J = 8.0 Hz, 4H), 7.46 (d, J = 8.0 Hz, 4H), 7.90 – 8.16 (m, 28H); ^{13}C NMR (CD_3OD) δ 34.7, 38.6, 42.9, 44.4, 50.8, 51.1, 51.1, 53.6, 61.6, 64.1, 128.9, 130.9, 136.7, 140.1, 174.7, 175.1; IR 3292 cm^{-1}, 1379 cm^{-1}, 1064 cm^{-1} MALDI-TOF MS Found: m/z 3489.67. Calcd for $C_{154}H_{280}N_{42}O_{44}S_2$: [$MH^+$], 3489.26.

Formation of the Dendron Thiol 8. A mixture of dendrimer disulfide **1** (53 mg, 0.015 mmol) and dithiothreitol (10 mg, 0.065 mmol) in methanol (10 mL) was stirred for 2 h under a N_2 atmosphere. After the usual work-up, the residue was purified by GPC to afford the dendron thiol **8** (53 mg, 0.028 mmol) as a thick oil in 95% yield: ^{1}H NMR (400 MHz, CD_3OD) δ 2.37 – 3.52 (m, 123H), 6,89 (d, J = 8.0 Hz, 2H), 7.19 (d, J = 8.0 Hz, 2H), 7.79 (brs, 2H), 7.97 (brs, 4H), 8.09 (t, J = 4.0 Hz, 8H). MALDI-TOF MS Found: m/z 1745.83. Calcd for $C_{77}H_{141}N_{21}O_{22}S$: [$MH^+$], 1746.14.

Conversion of Dendron Thiol 8 into Dendrimer 1. A solution of dendron **8** (50 mg, 0.027 mmol) in H_2O (10 mL) was oxidized by oxygen bubbling for 1 h. After the usual work-up, the residue was purified by GPC to afford dendrimer **1** (48 mg, 0.014 mmol) as a thick oil in 100% yield.

Formation of the Protected Dendron Thiol 9. To a solution of $NaBH_4$ (20 mg, 0.52 mmol) in ethanol (20 mL) was added dropwise a solution of dendrimer disulfide **1** (179 mg, 0.051 mmol) in ethanol (6 mL) under a N_2 atmosphere. After the mixture was stirred for 20 min, a solution of benzyl chloride (23 μL, 0.202 mmol) in ethanol (6 mL) was added dropwise. The mixture was stirred for 20 min. After the usual work-up, the residue was purified by GPC to given the dendron **9** (142 mg, 0.077 mmol) as a thick oil in 30% yeild: ^{1}H NMR (400 MHz, CD_3OD) δ 2.35 – 3.61 (m, 122H), 4.13 (s, 2H), 7.21 – 7.27 (m, 9H), 7.95 – 8.19 (m, 14H); ^{13}C NMR (CD_3OD) δ 34.7, 38.6, 39.6, 42.9, 43.1, 49.9, 50.8, 51.1, 51.1, 53,4, 53.6, 58.4, 61.6, 128.1, 129.4, 129.9, 130.6, 130.8, 136.3, 138.5, 139.1, 174.7, 175.1; IR 3300 cm^{-1}, 1377 cm^{-1}, 1067 cm^{-1}, MALDI-TOF MS Found: m/z 1835.78. Calcd for $C_{84}H_{147}N_{21}O_{22}S$: [$MH^+$], 1836.27.

This work was partly supported by the Association for the Progress of New Chemistry (ASPRONC), Grant-in-Aid for COE Research (No. 10CE2003), and Priority-Area-Research (B) (No. 12129205) by the Ministry of Education, Science, Sports and Culture. One of us (K. S.) thanks Sasagawa Scientific Research Grant from the Japan Science Society.

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13 Since λ_{max} of fluorescence emission of 2-anilinonaphthalene (0.03 mM) in the presence of dendron **8** or **9** (1.20 mM) shifts to 430 nm from 440 nm in water, dendrons **8** and **9** resemble in encapsulation properties.

14 The blue shift (by 16 nm) was observed for commercially available PAMAM dendrimer (G2.5). Therefore, the suitable arrangement of rigid cavity is necessary for the red shift as reported in Ref. 15.

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