Spherical Self-aggregates Formed with an Alanine-functionalized Porphyrin Derivative in Organic Solvent

Eun Jin Cho, Jeung Ku Kang, Sunwoo Kang,[†] Jin Yong Lee,[†,*] and Jong Hwa Jung[‡,*]

Department of Materials Science and Engineering, KAIST, Daejeon 305-701, Korea
[†]Department of Chemistry, Sungkyunkwan University, Suwon 440-746, Korea
[‡]Department of Chemistry and Research Institute for Natural Science, Gyeongsang National University, Chinju 660-701, Korea
[*]E-mail: jonghwa@gnu.ac.kr
Received August 23, 2006

Key Words : Self-Assembly, Porphyrin, Spherical, Alanine

It is well known that porphyrins and phthalocyanines tend to align into one-dimensional aggregates and, therefore, are of much interest in relation to the creation of novel supramolecular architectures, such as nanowires, discotic liquid crystals, and helical ribbon structures, etc. The major driving forces operating in these architectures are considered to be π-π stacking, intermolecular hydrogen bonding interactions, and/or van der Waals interactions.[1-8] For example, Shinkai and co-workers reported that self-assembled porphyrin derivatives having amide groups efficiently form fiber structures by π-π stacking as well as through intermolecular hydrogen bonding interactions in organic solvents.[9] Particularly, the hydrogen bond forming groups in porphyrin derivatives play an important role in the formation of the final aggregation mode. Therefore, we designed an alanine-functionalized porphyrin derivative in order to form self-assembled superstructures through intermolecular hydrogen bonding interactions. The alanine-functionalized porphyrin is constructed by introducing four alkyl alanine residues onto the macrocyclic skeleton. We report here the preparation and the aggregate formation of **1** in organic solvent, as demonstrated by field-emission scanning electron microscopy (FE-SEM), energy-filtered transmission electron microscopy (EF-TEM), confocal laser scanning microscopy (CLSM), FT-IR, and fluorescence spectroscopy studies.

Alanine-functionalized porphyrin **1** was synthesized in six steps as shown in Scheme 1. The alanine ethyl ester was added to a solution of the corresponding tetraphenyl carboxy acid **3**. After de-ethylation of **5**, treatment with **6** and diaminoacetylene **10** afforded the desired product **1** as a dark brown powder. Also, compound **2** without alanine moieties was prepared as reference.

The aggregation behavior of **1** was investigated in organic solvents such as toluene or chloroform by using several microscopic, and spectroscopic methods. In order to obtain visual insights into the aggregation mode, we observed the superstructure of self-assembled **1** and **2** by FE-SEM and EF-TEM. Figure 1 shows FE-SEM and EF-TEM images of the self-assembled **1** formed in toluene. The FE-SEM and EF-TEM images of unstained samples in toluene show a spherical structure with uniform diameter of ≈600 nm, which have no hollow cavity. In contrast, compound **2** which lacked alanine moieties did not reveal any morphology due to high solubility in organic solvents. These results indicate that the alanine moiety plays a critical role in forming the self-assembled spherical structure. Also, the findings suggest that the self-assembled spherical structure of **1** might be induced mainly by strong intermolecular hydrogen bonding interactions between alanine and alanine moieties in organic solvents.

Hydrogen bonding interactions of alanine moieties of **1** in toluene were evaluated by FT-IR measurements and compared with those of **2** without the alanine moiety. The absorption frequencies originating from the N-H deformation band and from the C=O stretching vibrations of **1** shifted to higher and lower wavenumbers (N-H: 1534 and C=O: 1623 cm^{-1}), as compared to the comparable absorption frequencies for the reference compound **2** (N-H: 1556 and C=O: 1638 cm^{-1}), indicating the formation of hydrogen bonds for almost all the amide residues of **1** (Figure S1).

To have an insight on the role of hydrogen bonding between amide groups in the self-assembly of **1**, we simplified the molecule **1** by replacing the alkyl chain after the amide group with methyl. It is clearly shown in Figure S2 that the hydrogen bonding between amide groups play an important role in growing by self-assembly. In particular, all the four chains seem to be involved in the intermolecular hydrogen bonding in the self-assembly. The thermodynamic stability of uuuu, uudd, and udud structures is virtually equivalent (within 0.12 kcal/mol) at the AM1 calculations (see calculation results and Scheme S1 in Supporting Information). In our experiment, the spherical self-assembled structure was

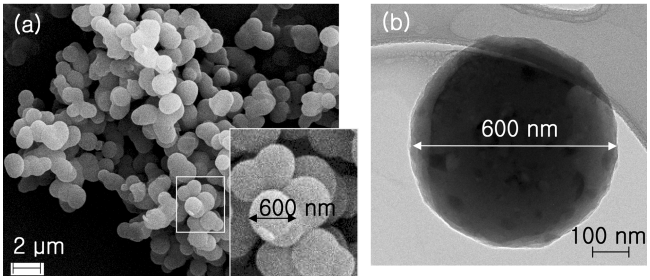
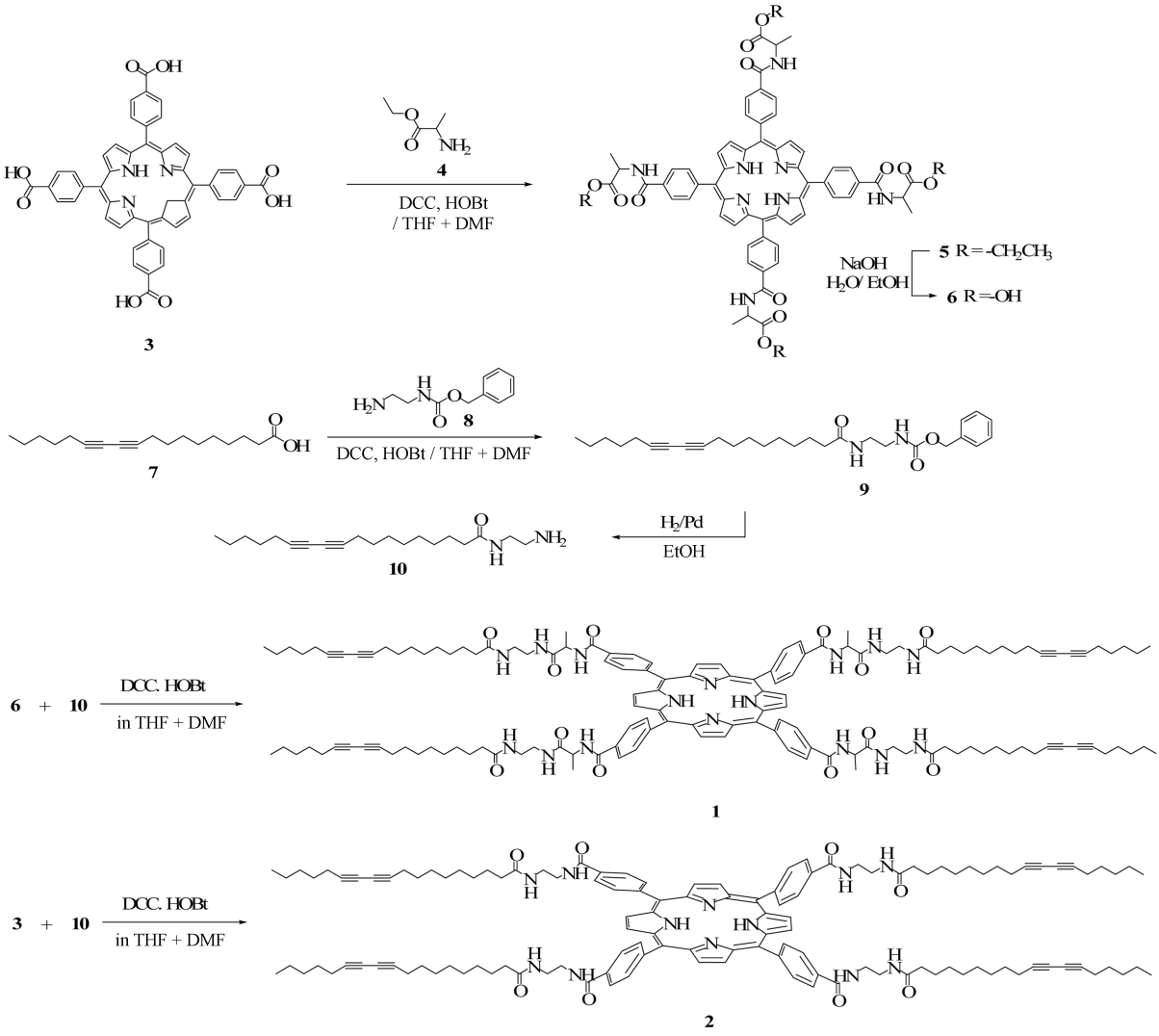


Figure 1. (a) FE-SEM and (b) EF-TEM images of self-assembled **1** (2.5 wt %) in toluene.



Scheme 1. Synthetic routes for alanine-functionalized porphyrin derivatives.

observed, thus, the building block is expected to be "udud" type structure because "udud" is the only structure that can result a 3-dimensional network in self-assembly (Scheme S1).

To confirm the molecular packing structure, we measured the powder-X-ray diffraction pattern of the self-assembled **1** (Figure S3). The observed strong high-ordered reflection peak at 1.85° indicates the existence of a well-organized layer structure. The *d*-spacing is calculated from the Bragg equation to be 47.7 Å, which is estimated from CPK space-filling model. This result suggests that the side chains around the alanine residues of **1** were well-assembled and constructed in a layer structure through inter- and intra-molecular hydrogen bonds. Also, the long alkyl chain group of **1** forms the hydrophophic interaction.

Dye loading of the self-assembled **1** is conducted by liquid-liquid phase transfer as shown in Figure S4. In liquid-liquid phase transfer, an aqueous solution of the dye is transferred into a layer of the self-assembled **1**.

To confirm encapsulation of rhodamine B molecules into the self-assembled **1**, the rhodamine B adsorbed outside on the surface of the self-assembled spherical structure **1** was removed by washing with water. Then, we observed CLSM images by excitation with 554 nm. As shown in Figure 2, self-assembled **1** loaded with rhodamine B shows a spherical structure. This observation supports the notion that the majority of rhodamine B present in the self-assembled nanospherices is physically adsorbed. Also, the EF-TEM

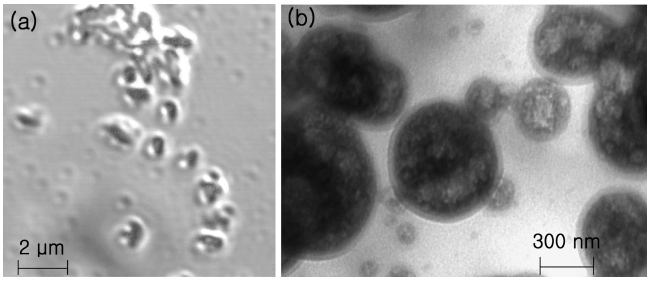


Figure 2. (a) CLSM and (b) TEM images of self-assembled **1** encapsulated with rhodamine B in toluene.

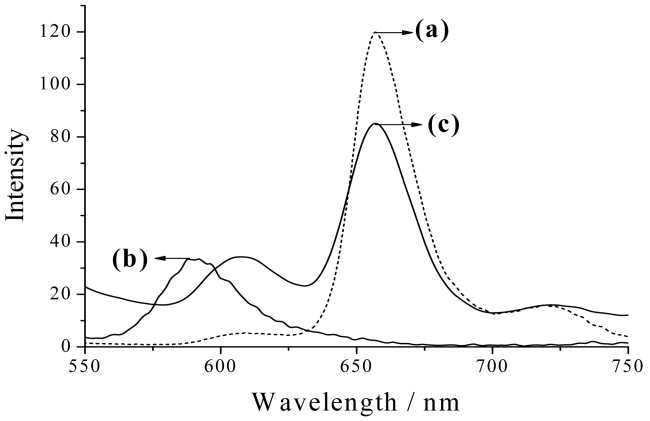


Figure 3. Fluorescent spectra of (a) self-assembled **1**, (b) rhodamine B, and (c) **1**/rhodamine B in toluene.

image of the self-assembled **1** encapsulated with rhodamine B revealed a spherical structure with ≈600 nm diameter, indicating that after loading the rhodamine B molecules, the spherical structure of the self-assembled **1** maintains stably by π-π stacking.

Furthermore, clear evidence for encapsulation of rhodamine B in the self-assembled spheres **1** was obtained by fluorescence spectroscopy (Figure 3). The fluorescence emission spectrum of the self-assembled **1** encapsulated with rhodamine B in toluene shows peaks at 607 and 657 nm. Particularly, in the spectrum of nanoparticle encapsulated with rhodamine B, the emission intensity at 657 nm was less than that of **1** without rhodamine B, indicating that the rhodamine B molecules are intercalated between porphyrin and porphyrin moieties by π-π stacking. In addition, the rhodamine B fluorescence in the self-assembled **1** was observed to shift to longer wavelength in comparison to rhodamine B itself. The finding suggests that the rhodamine B was efficiently stacked between the porphyrin moieties.

In summary, porphyrin derivative **1** having four alanine moieties was found to form spherical self-aggregates in toluene as confirmed by EF-TEM and FE-SEM studies. The polar alanine groups of **1** play key roles for the formation of the aggregate. Also, the self-assembled aggregates have successfully encapsulated the guest molecule rhodamine B by π-π stacking. We believe that this aggregate itself may have interesting applications as well, since multimolecular micelles are being studied for their use in fields such as drug delivery, diagnosis, separation technology, and optotechnology. To this purpose, we are currently investigating the structure of these aggregates in more detail, as well as the incorporation of functional additives into the aggregates.

Experimental Section

Compound **4**[10] and **8**[11] were prepared from the adaptation of the reported procedures.

Compound 1. Compound **6** (0.2 g, 0.19 mmol) was dissolved in THF/DMF (10 : 1) and the solution cooled to 0 °C. Compound **10** (0.3 g, 0.93 mmol), 1, 3-Dicyclohexylcarbodiimide (DCC, 0.19 g, 0.93 mmol), and 1-Hydroxybenzotriazole hydrate (HOBt, 0.13 g, 0.93 mmol) were then added successively. The reaction mixture was refluxed for 15 h. After removing the solvent, the residue was taken up in $CHCl_3$ and washed with water. The organic solvent was evaporated to dryness. The residue was purified by a silica-gel column eluting with $CHCl_3$/MeOH to give compound **1** in yield 10% as dark brown solid. M.p. 230 °C; ^{1}H-NMR ($CDCl_3$ + MeOD-d_1): δ = 8.74 (m, 8H, ArH) 8.34 (m, 16H, ArH), 4.85 (br s, 4H, -NH), 3.62 (m, 16H, -CH_2CH_2-), 2.32 – 0.69 (m, 72H, -CH_2CH_2- & -CH_3).

Compound 2. TCPP [Tetrakis(4-carboxyphenyl)porphine] **3** (0.50 g, 0.63 mmol) was dissolved in THF/DMF (10 : 1) and the solution cooled to 0 °C. Compound **10** (1.0 g, 3.16 mmol), DCC (0.65 g, 3.16 mmol), and HOBt (0.43 g, 3.16 mmol) were then added successively.

A solution of tetrakis(4-carboxyphenyl)porphine **3** (0.50 g, 0.63 mmol), compound 10 (1.0 g, 3.16 mmol), DCC (0.65 g, 3.16 mmol), and HOBt (0.43 g, 3.16 mmol) were refluxed for 20 h in THF/DMF (10 : 1). The reaction mixture was refluxed for 20 h. After removing the solvent, the residue was taken up in $CHCl_3$ and washed with water. The organic solvent was evaporated to dryness. The residue was purified by a silica-gel column eluting with $CHCl_3$/MeOH to give compound **2** in yield 20% as dark brown solid. M.p. 227 °C; ^{1}H-NMR ($CDCl_3$): δ= 8.72-8.49 (m, 16H, ArH), 8.39 (d, J = 7.5 Hz, 8H, -ArH), 6.36 (br s, 2H, NH), 6.19 (br s, 2H, NH), 4.91 (bs, 4H, NH), 4.22 (m, 4H, -CH_2CH_2-), 3.66 (m, 12H, -CH_2CH_2-), 2.36-0.79 (m, 60H, -CH_2CH_2- & -CH_3).

Compound 5. Tetrakis(4-carboxyphenyl)porphine (0.5 g, 0.63 mmol) was dissolved in THF/DMF (30 : 3) and the solution cooled to 0 °C. 1, 3-Dicyclohexylcarbodiimide (DCC, 0.59 g, 2.85 mmol), 1-Hydroxybenzotriazole hydrate (HOBt, 0.38 g, 2.85 mmol), Compound **4** (0.4 g, 2.85 mmol) and triethylamine (0.2 mL, 0.28 mmol) were then added successively. The reaction mixture was refluxed for 24 h. The THF was then evaporated, and the residuce dissolved in water. The precipitated solid was filtered and purified by a silica-gel column eluting with $CHCl_3$/MeOH to give compound **5** in yield 75% as dark brown solid. M.p. 270 °C; ^{1}H-NMR ($CDCl_3$): δ = 8.84 (s, 4H, ArH), 8.72 (d, J = 7.5 Hz, 6H, ArH), 8.64 (br s, 2H, NH), 8.49 (m, 4H, ArH), 8.35-8.21 (m, 10H, ArH), 4.99 (q, J = 6.9 Hz, 4H, -HNCH(CH_3)-), 4.35 (q, J = 7.2 Hz, 8H, -CH_2CH_3), 3.93 (d, J = 6.9 Hz, 4H, -NH), 1.71 (t, J = 7.2 Hz, 12H, -CH_3), 1.41 (q, J = 7.2 Hz, 12H, -CH_3); ^{13}C-NMR (300 MHz, $CDCl_3$): δ = 173.70 (C=O), 167.18 (C=O), 145.66 (CH), 134.85 (CH), 133.73 (CH), 128.74 (CH), 125.80 (CH), 119.55 (CH), 62.08 (CH), 52.98 (CH), 49.08 (CH), 49.00 (CH), 19.05 (CH), 18.98 (CH), 14.45 (CH).

Compound 6. Compound **5** (1.0 g, 0.84 mmol) was dissolved in tetrahydrofuran (20 mL). The solution was cooled to 0 °C and then aqueous sodium hydroxide solution (0.3 g in H_2O, 1.0 mL) was added. The reaction was stirred under nitrogen for 24 hours. The solvent was removed by rotary evaporation, water was added (30 mL) and then the mixture was acidified to pH 3 with aqueous sodium hydro-

gen sulfate. The product was extracted with ethylacetate and then washed with water and brine. The resulting solution was dried ($MgSO_4$) and then the solvent was removed by rotary evaporation to dryness. Diethyl ether (20 mL) was added and then solvent was removed from the mixture by rotary evaporation to give product (0.55 g, 0.30 mmol, 76%). M.p. 280-282 °C; ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 8.96 (d, 4H, J = 7.2 Hz, NH), 8.87 (s, 8H, ArH), 8.36 (s, 16H, ArH), 4.61 (q, 4H, -NHC(CH_3)H-), 1.53 (d, 12H, J = 7.2 Hz, -CH_3); ^{13}C-NMR (300 MHz, DMSO-d_6): δ= 174.27 (C=O), 166.09 (C=O), 144.01 (CH), 134.21 (CH), 134.11 (CH), 126.10 (CH), 110.20 (CH), 92.97 (CH), 48.40 (CH), 30.67 (CH), 17.02 (CH), 15.81 (CH).

Compound 9. 10, 12-Octadecadiynoic acid (compound **7**, 0.5 g, 1.81 mmol) was dissolved in THF/DMF (20 : 1) under a nitrogen atmosphere. The solution was maintained at 0 °C with an ice bath. The dicyclohexylcarbodiimide (DCC, 0.383 g, 1.86 mmol) and HOBt (0.022 g, 0.186 mmol), and compound **8** (0.42 g, 2.17 mmol) were then added, the reaction mixture being stirred for 24 h at room temperature. After removing the solvent, the residue was taken up in $CHCl_3$ and washed with water. The organic solvent was evaporated to dryness. The residue was purified by a silica-gel column eluting with EA/*n*-Hexane to give compound **9** in yield 50% as white solid. M.p. 114 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 7.80 (m, 1H, ArH), 7.46 (m, 1H, ArH), 7.34 (m, 3H, ArH), 5.09 (s, 2H, -$CH_2C_6H_5$), 3.35 (m, 4H, -$NHCH_2CH_2NH$-), 2.23 (m, 4H, -CH_2-), 2.14 (t, 2H, J = 7.5 Hz, -CH_2-), 1.54-1.25 (m, 20H, -CH_2-), 0.89 (t, 3H, J = 6.9 Hz, -CH_2CH_3).

Compound 10. Compound **9** (2.0 g, 4.42 mmol) was dissolved in a mixture of ethanol (20 mL). Then, 10% Pd-C (25 mg) was added to the solution. Hydrogen gas was introduced into the mixed solution for 24 h at room temperature. The reaction mixture was filtered to remove Pd-C and the filtrate was evaporated *in vacuo* to dryness. Yield 70%, white solid. M.p. 109 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 4.17 (br s, 2H, -$NHCH_2CH_2$-), 3.48 (br s, 2H, -$CH_2CH_2NH_2$), 1.97-0.89 (m, 31H, -CH_2CH_2- and -CH_3).

Acknowledgements. This work was supported in part by the KOSEF (F01-2004-10061-0) and KRF (KRF-2005-005-J09703).

Supporting Information Available: FT-IR spectra of the self-assembled **1** and **2** and calculation results, XRD diffraction pattern and dye loading method of the self-assembled **1**. This material is available *via* the Internet at *http://www.kcsnet.or.kr/bkcs*.

References

1. (a) Van Nostrum, C. F.; Picken, S. J.; Schouten, A.-J.; Nolte, R. J. M. *J. Am. Chem. Soc.* **1995**, *117*, 9957. (b) Schenning, A. P. H.; Benneker, F. B. G.; Geurts, H. P. M.; Liu, X. Y.; Nolte, R. J. M. *J. Am. Chem. Soc.* **1996**, *118*, 8549. (c) Van Nostrum, C. F.; Nolte, R. J. M. *Chem. Commun.* **1996**, 2385.
2. (a) Fuhrhop, J.-H.; Demoulin, C.; Boettcher, C.; Köning, J.; Siggel, U. *J. Am. Chem. Soc.* **1992**, *114*, 4159. (b) Bindig, U.; Schulz, A.; Fuhrhop, J.-H. *New J. Chem.* **1995**, *19*, 427 and references therein.
3. (a) Smolenyak, P.; Peterson, R.; Nebesny, K.; Torker, M.; O'Brien, D. F.; Armstrong, N. R. *J. Am. Chem. Soc.* **1999**, *121*, 8628. (b) Drager, A. S.; Zangmeister, R. A. P.; Armstrong, N. R.; O'Brien, D. F. *J. Am. Chem. Soc.* **2001**, *123*, 3595.
4. (a) Kimura, M.; Kitamura, T.; Muto, T.; Hanabusa, K.; Shirai, H.; Kobayashi, N. *Chem. Lett.* **2000**, 1088. (b) Kimura, M.; Muto, T.; Takimoto, H.; Wada, K.; Ohta, K.; Hanabusa, K.; Shirai, H.; Kobayashi, N. *Langmuir* **2000**, *16*, 2078. (c) Kimura, M.; Wada, K.; Ohta, K.; Hanabusa, K.; Shirai, H.; Kobayashi, N. *J. Am. Chem. Soc.* **2001**, *123*, 2438.
5. (a) Imada, T.; Murakami, H.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1994**, 1557. (b) Arimori, S.; Takeuchi, M.; Shinkai, S. *J. Am. Chem. Soc.* **1996**, *118*, 245. (c) Arimori, S.; Takeuchi, M.; Shinkai, S. *Supramol. Sci.* **1998**, *5*, 1.
6. Nagata, N.; Kugimiya, S.; Kobuke, Y. *Chem. Commun.* **2001**, 689.
7. Kawano, S.-I.; Tamaru, S.-I.; Fujita, N.; Shinkai, S. *Chem. Eur. J.* **2004**, *10*, 343. and references therein.
8. Jung, J. H.; Rim, J. A.; Lee, S. J.; Lee, H.; Park, S. M.; Kim, B. H. *Bull. Korean Chem. Soc.* **2005**, *26*, 34.
9. (a) Kichida, K.; Fujita, N.; Sada, K.; Shinkai, S. *J. Am. Chem. Soc.* **2005**, *127*, 7298. (b) Shirakawa, M.; Fujita, N.; Shinkai, S. *J. Am. Chem. Soc.* **2005**, *127*, 4164. (c) Shirakawa, M.; Fujita, N.; Shinkai, S. *J. Am. Chem. Soc.* **2003**, *125*, 9902.
10. Jakusch, T.; Dörnyei, Á.; Correia, I.; Rodrigues, L. M.; Tóth, G. K.; Kiss, T.; Costa Pessoa, J.; Marcão, S. *Eur. J. Inorg. Chem.* **2003**, 2113.
11. Aoi, K.; Itoh, K.; Okada, M. *Macromolecules* **1997**, *30*, 8072.