Notes

Structural and Thermosensitive Properties of Cyclotriphosphazenes with Poly(ethylene glycol) and Amino Acid Esters as Side Groups

Sang Beom Lee,[†] Soo-Chang Song,*[,†] Jung-Il Jin,[‡] and Youn Soo Sohn*[,§]

Inorganic Chemistry Laboratory, Korea Institute of Science and Technology, Seoul 130-650, Korea, Department of Chemistry, Korea University, Seoul 136-701, Korea, and Department of Chemistry, Ewha Womans University, Seoul 120-750, Korea

Received April 13, 2001
Revised Manuscript Received July 23, 2001

Introduction

Polymers with thermosensitivity have a great potential for applications in multidisciplinary areas such as membranes,[1,2] drug delivery,[3,4] solute separation,[5] and enzyme activity control.[6] Such applications call for some specific thermosensitive properties suitable for each purpose. In particular, polymers for biomedical applications require precise and reproducible thermosensitive properties, but most of the polymers are polydisperse and as such difficult to reproduce with the exactly the same hydrophilic and hydrophobic compositions. Therefore, the development of macromolecules with both monodisperse molecular weight and precise hydrophilic/hydrophobic balance may be a key step toward practical application to targeting drug delivery systems (DDS).

Poly(ethylene glycols) (PEGs) are representative thermosensitive polymers by themselves,[7] but these polymers have also been incorporated into polyphosphazenes for the preparation of stimulus-sensitive polymers[8−10] and polymer electrolyte.[11−13] In particular, a new class of thermosensitive and biodegradable poly(organophosphazenes) were synthesized by the authors. However, all these thermosensitive polymers are polydisperse and thus difficult to control exactly their lower critical solution temperature (LCST), as mentioned above. Also, macrocyclic substituted cyclotriphosphazenes have been described earlier by Brandt et al.[14,15] The first bis-transannular cyclotriphosphazene derivatives were obtained by regioselective sodium cation-assisted reactions of a PEG. The study was meaningful in that a new class of cyclotriphosphazenes could be synthesized by regiocontrol of hexachlorocyclotriphosphazene with a low molecular PEG.

However, no cyclotriphosphazene with thermosensitive properties was reported until we communicated a successful synthesis of thermosensitive cyclotriphosphazenes by regio- and stereoselective stepwise substitutions of the chlorotrimer with hydrophilic alkoxypoly(ethylene glycol) (APEG) and hydrophobic amino acid esters (AEE).[16] Here we present a detailed description of the structural aspects and thermosensitive properties of these phosphazene trimers, together with some additional findings. For structural characterization we have monitored by ^{31}P NMR spectroscopy the initial substitution reaction of the chlorotrimer with a PEG depending on the reaction temperature. Also, studies were undertaken to examine their LCST properties as well as their hydrolytic degradability.

Experimental Section

Cyclic Trimers. Among the trimeric derivatives bearing methoxypoly(ethylene glycol) and amino acid esters as side groups previously synthesized,[16] $N_3P_3(MEE)_3(GlyBz)_3$ (**1**), $N_3P_3(MEE)_3(AspEt_2)_3$ (**2**), $N_3P_3(MPEG350)_3(GlyBz)_3$ (**3**), $N_3P_3(MPEG350)_3(AspEt_2)_3$ (**4**), $N_3P_3(MPEG350)_3(AspBz_2)_3$ (**5**), $N_3P_3(MPEG550)_3(AspBz_2)_3$ (**6**), and $N_3P_3(MPEG750)_3(AspBz_2)_3$ (**7**) were employed for the present study.

Materials. Hexachlorocyclotriphosphazene (Aldrich) was used without further purification. Benzyl ester of glycine and ethyl or benzyl ester of L-aspartic acid were prepared by the literature method.[17] Methoxypoly(ethylene glycol) with molecular weights of 350 (MPEG350) and 750 (MPEG750) and 2-(2-methoxyethoxy)ethanol (MEE) were dried azeotropically with benzene, followed by vacuum-drying, and then stored over molecular sieve 3A. Tetrahydrofuran (THF) and triethylamine were dried by refluxing over sodium metal and barium oxide, respectively, and then distilled under a nitrogen atmosphere. Triton X-100 and Pluronic L64 were used as received from Aldrich and BASF, respectively. Acetic acid (Junsei) and anhydrous sodium acetate (Kanto) were used as received for the preparation of buffer solutions.

Measurements. ^{31}P NMR spectra were recorded using a Varian Gemini-300 spectrometer operating at 121.4 MHz. All the spectra were measured in anhydrous acetone-d_6 using triphenyl phosphate as an external standard. The LCST of the aqueous trimer solutions was measured by both turbidity and UV−vis spectroscopic methods as previously described.[10] The absorbance of the trimer solutions was measured at 600 nm at intervals of 1 °C.

Cloud Point (CP) Separation. A trimer solution of 5 mL (5 wt % in water) was pipetted into a 10 mL long tapered centrifuge tube. To this was added a trifluoroethanol (2.0−2.5 M) to precipitate the content due to its lowered LCST. The tube was moved to an ice bath to make a transparent solution, which was incubated in a thermostated shaking water bath for 15 min at the desired temperature (20−30 °C). The trimer solution was then centrifuged at 3500 rpm for 5 min, after the centrifuge rotor was heated to the desired temperature just before centrifugation. The clear aqueous layer was then decanted off to obtain a trimer-precipitated phase which adhered to the bottom of the tube. Trifluoroethanol was then removed from the precipitate by evaporation, and then the trimer rich phase was freeze-dried.

In Vitro Hydrolytic Degradation of Trimers. Time-dependent degradation of the thermosensitive trimers was examined in different pH buffer solutions at 36 °C. The trimers (50 mg) were dissolved in 1 mL of 0.5 M buffer solutions (acetate buffer of pH 5, tris buffer of pH 7.4, and carbonate buffer of pH 10), which were incubated in a water bath at 36 °C. Time-dependent hydrolytic behavior of the trimers was examined by ^{31}P NMR spectroscopy.

[†] Korea Institute of Science and Technology.
[‡] Korea Unversity.
[§] Ewha Womans University.

10.1021/ma010648b CCC: $20.00
Published on Web 09/12/2001

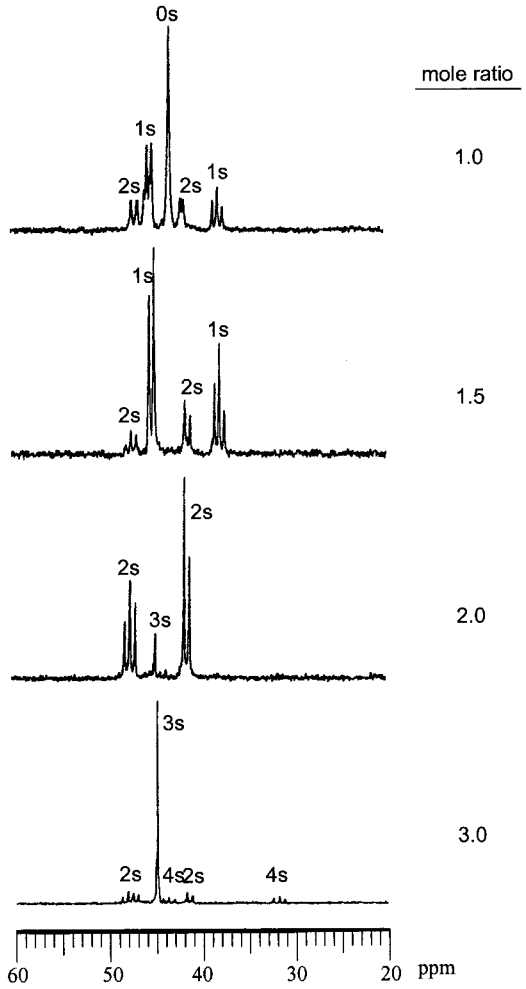
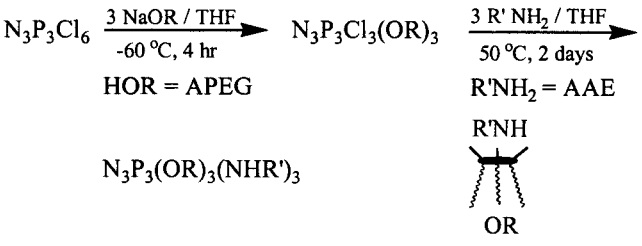


Figure 1. ^{31}P NMR spectra of the trimeric isomers with various mole ratios of MEE (0s, $N_3P_3Cl_6$; 1s, $N_3P_3(MEE)_1Cl_5$; 2s, $N_3P_3(MEE)_2Cl_4$; 3s, $N_3P_3(MEE)_3Cl_3$; 4s, $N_3P_3(MEE)_4Cl_2$.

Results and Discussion

Synthesis and Structural Study. The present trimeric compounds were prepared by stepwise substitution of hexachlorocyclotriphosphazene with APEG and AAE as shown in our previous work:[16]



For the structural study of cyclotriphosphazene derivatives, ^{31}P NMR spectroscopy is one of the most powerful tools, since most critical structural information are available from the chemical shifts and spin−spin coupling data of their ^{31}P NMR spectra.[18,19]

Figure 1 shows the ^{31}P NMR spectra monitored during the substitution reaction of $N_3P_3Cl_6$ with MEE at −60 °C depending on the mole ratio of the substituent. Unreacted hexachlorocyclotriphosphazene shows a sharp singlet of ^{31}P resonance at 43 ppm, but addition of 1 mol of MEE to the chlorotrimer gave a mixture of partially substituted products $N_3P_3(APEG)_xCl_{6-x}$ including monosubstituted (38 and 46 ppm) and disubstituted isomers (42 and 48 ppm). Increasing the content of MEE, the peak at 43 ppm disappeared whereas those of the mono- and disubstituted isomers increased in intensity. Further increasing the mole ratio of MEE to the trimer up to 3, the peak at 45 ppm became predominant with almost disappearance of the peaks

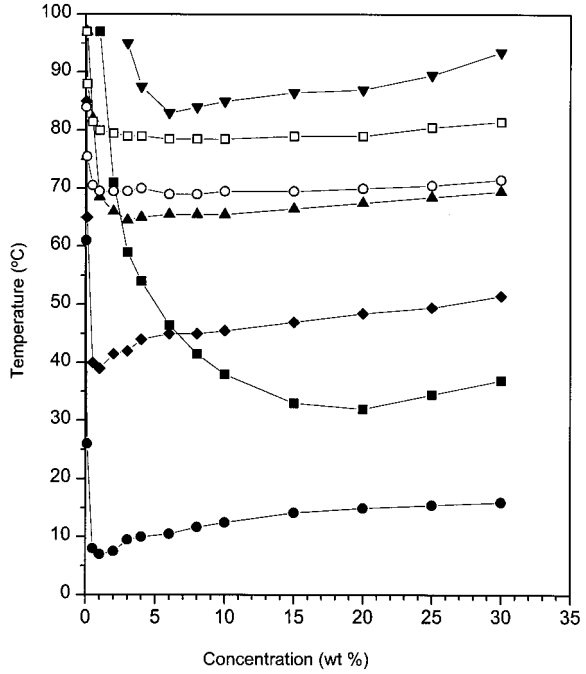


Figure 2. Concentration-dependent LCST behaviors of trimers **1** (●), **2** (■), **3** (▲), **4** (▼), **5** (◆), **6** (○), and **7** (□).

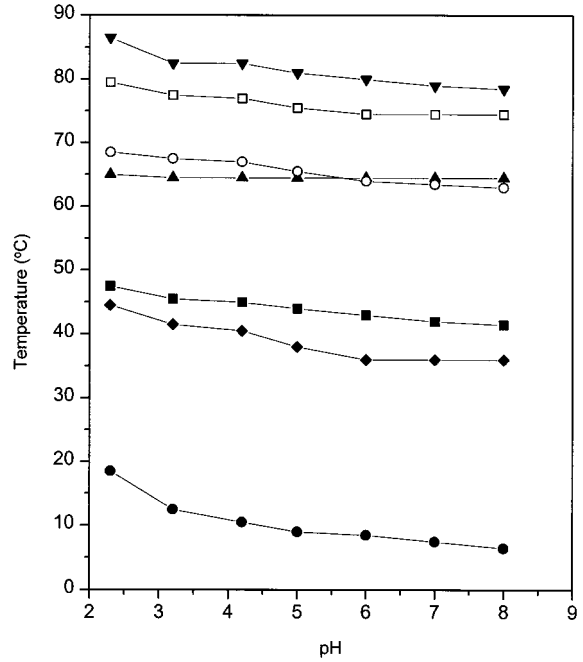


Figure 3. The pH effect on the LCST of trimers **1** (●), **2** (■), **3** (▲), **4** (▼), **5** (◆), **6** (○), and **7** (□).

Table 1. Compositions of Isomers of $N_3P_3((MEE)_3Cl_3$ Depending on the Reaction Temperature

reaction temp (°C)	composition of isomers
−60	cis-2,4,6 (82%), a mixture of cis-2,4 and cis-2,2,4,6 (18%)
25	cis-2,4,6 (16%), a mixture of cis-2,4, cis-2,2,4,6, and trans-2,4,6 (84%)

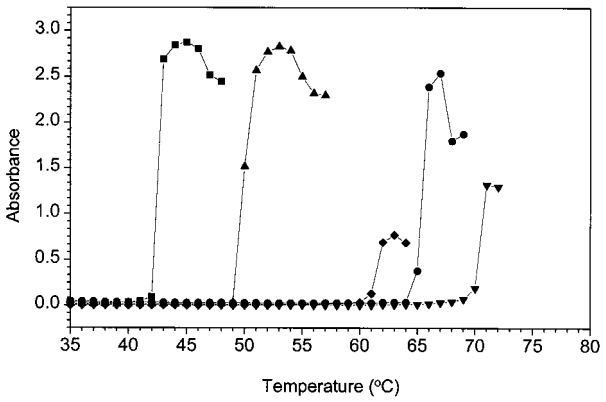


Figure 4. Phase transition behaviors of trimers **3** (●) and **5** (■) and NP(MPEG350)$_{1.00}$(AspEt$_2$)$_{1.00}$ (▲), Triton X-100 (▼), and Pluronic L64 (◆).

due to the mono- and disubstituted isomers. The peak at 43 ppm is attributed to the phosphorus resonance of the ring Cl−P−Cl unit in $N_3P_3Cl_6$. The signal from the −O−P−Cl unit in N_3P_3(MPEG350)$_3Cl_3$ is almost 2 ppm downfield shifted compared to that of the Cl−P−Cl unit. Such a change in the ^{31}P resonance from a singlet of $N_3P_3Cl_6$ to a singlet of N_3P_3(MPEG350)$_3Cl_3$ clearly indicates that the nucloephilic substitution by MEE at low temperature leads to almost exclusively the cis-nongeminal isomer. After stepwise substitution of N_3P_3(MPEG350)$_3Cl_3$ with L-aspartic acid ethyl ester, the final product was further purified by CP separation.

As mentioned above, the initial substitution of the chlorotrimer with MEE gave the cis-2,4,6 isomer in a high yield at low reaction temperature, but at room temperature a mixture of cis- and trans-isomers was obtained as shown in Table 1. Such results indicate that the selectivity in the substitution reaction of hexachlorocyclotriphosphazene with MEE is strongly dependent on the reaction temperature.

Thermosensitive Properties. The LCST of the present trimeric derivatives in aqueous solution was reported earlier.[16] In the present study, the concentration effect on the LCST of these trimers was determined in the concentration range 0.01−30 wt % of the polymers in aqueous solution, and the results are shown in Figure 2. Surprisingly, trimers **1**, **5**, **6**, and **7** showed their LCST at very low concentration even less than 0.5 wt %, in contrast to the corresponding poly(organophosphazenes) bearing the same substituents MPEG and

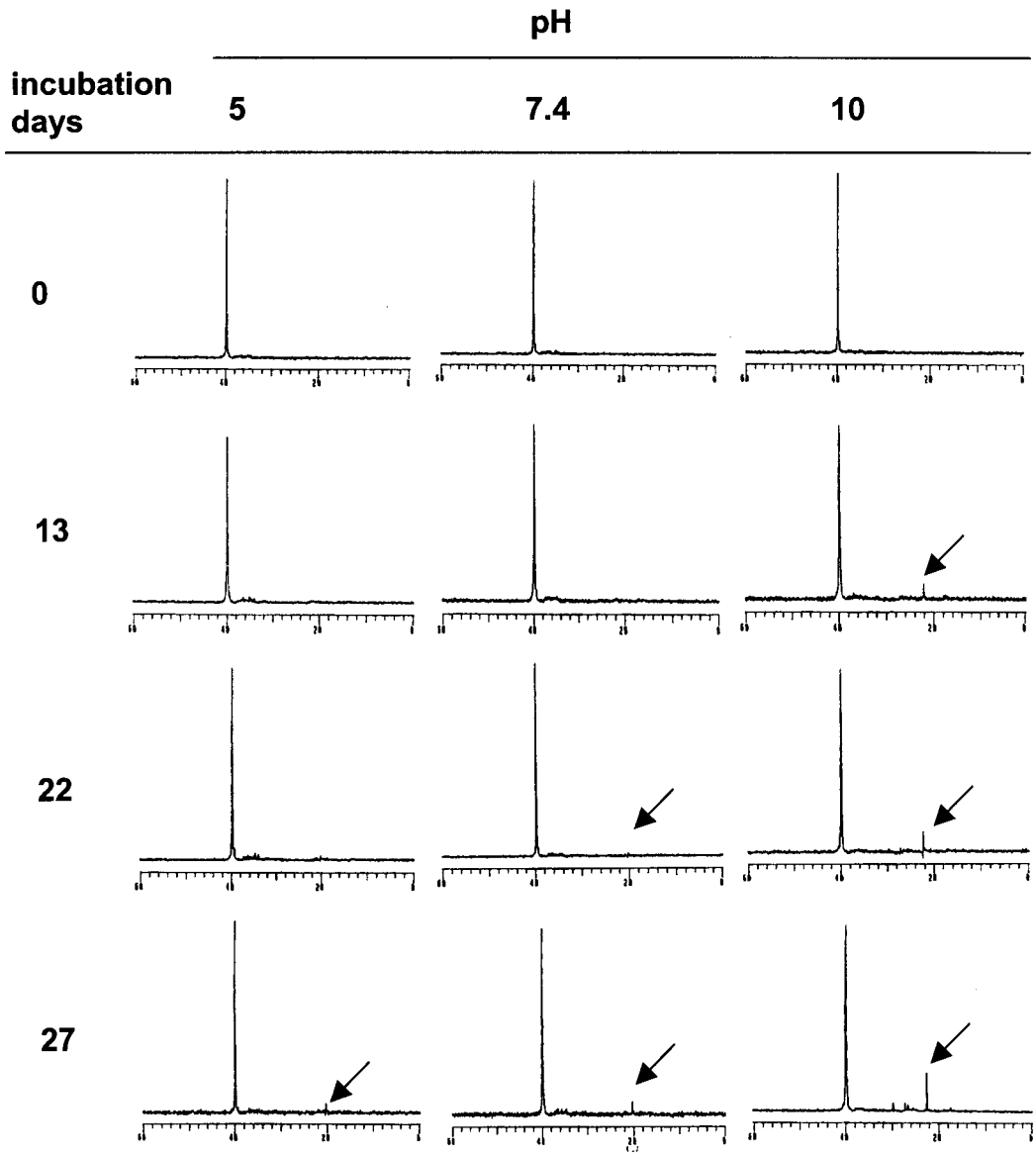


Figure 5. ^{31}P NMR spectra of trimer **2** before and after incubatuin in different buffer solutions at 36 °C.

AAE which exhibited their LCSTs usually at above 2 wt %.[10] These trimeric derivatives have three hydrophobic groups on the same side from the ring plane, which may afford easier and stronger intermolecular hydrophobic interaction among the trimeric molecules to form giant aggregates. Therefore, the trimeric derivatives with a good conformational mobility compared with the linear polymer bearing the same side groups seem to exhibit their LCST properties even at lower concentrations. On the other hand, trimers **2** and **4** showed lower LCSTs at relatively high concentrations of 20 and 5 wt %, respectively. The reason seems to be due to the smaller ethyl ester group of aspartic acid of these trimers as the hydrohobic part, compared with the benzyl ester of the same amino acid of other trimers.

Figure 3 shows the pH effect on the LCST of trimers in acetate buffer solutions. When the pH of the aqueous trimer solutions was increased, their LCSTs were found to decrease apparently in the acidic region (pH = 2−4) but showed only a slight pH dependence in other pH region, which is similar to the MPEG-based polymers.[20] The LCST increase in the acidic pH range seems to be due to ionization of the amino group of AAE.

Rate of Phase Transition. The LCST value of thermosensitive materials is an important factor to determine the feasibility of their practical applications. The LCST value is usually governed by the structure, polarity, and orientation of the molecules in aqueous media. Especially, thermosensitive materials for biomedical applications should be considered on the basis of the biological temperature. In addition, the rate of phase transition may be another important factor when the thermosensitive materials are considered as a carrier for local drug delivery using the biological temperature of living organisms.

Figure 4 shows the absorbances of trimers **3** and **5** determined as a function of temperature by UV−vis spectroscopy at 600 nm to compare with other thermosensitive polyphosphazenes and nonionic surfactants. Increasing the temperature of the polymer solutions, their absorbance increased sharply at around their LCST, which was defined as the temperature where 50% change of the absorbance occurred. Interestingly, trimers **3** and **5** exhibited larger absorbance changes ($\delta A/\delta T$) than the polymeric thermosensitive materials such as $[NP(MPEG350)_{1.00}(GlyBz)_{1.00}]_n$,[10] Triton X-100, and Pluronic L64: trimer **5** showed approximately 2 and 4 times larger absorbance change than Triton X-100 and Pluronic L64, respectively. Also, trimer **3** with MPEG350 and glycine benzyl ester as side groups exhibited 1.5 times larger absorbance change than the corresponding polymer with the same substituents $[NP(MPEG350)_{1.00}(GlyBz)_{1.00}]_n$. Such results indicate that the LCST of the present thermosensitive materials is influenced significantly by the hydrophilic/hydrophobic balance as well as by their molecular structure. As mentioned above, the present trimers have three hydrophobic groups in one direction from their ring plane, which can afford efficient intermolecular hydrophobic interaction among the trimeric molecules, resulting in easier aggregation from their solutions, in contrast to Triton X-100 (Figure 5d), for example, which is a linear molecule with one hydrophobic group and one hydrophilic group. After all, the trimers are easy to form giant aggregates, which makes a fast phase transition in solution.

Hydrolytic Behavior. To study hydrolytic properties of the present thermosensitive trimers, they were dissolved in different buffer solutions (pH 5, pH 7.4, and pH 10) at 36 °C, and their hydrolysis process was monitored by ^{31}P NMR spectroscopy. Trimer **2** showed significantly faster hydrolysis in the basic buffer solution than the acidic and neutral solutions as shown in Figure 5. The hydrolytic behaviors of the trimers substituted with amino acid esters and MEE can be explained in terms of ring opening of the trimers, hydrolysis of the amino acid ester, and the amino acid−phosphorus bond cleavage. The initial ring opening was observed in the buffer solution of pH 10 in about 13 days. The rate of the ring opening in the buffer solutions increased in the order of neutral < acidic < basic solution. Interestingly, from ^{31}P and ^{1}H NMR monitoring, the ring-opening degradation of the trimers was found to proceed simultaneously with cleavage of the ester group from AAE and detachment of AAE from the phosphorus atom. Such a result means that the ring opening and detachment of AAE from the phosphorus atom are strongly affected by hydrolysis of AAE generating the free carboxylic acid group, which induces the acid-catalyzed degradation.[20] Thus, the hydrolysis of the present trimers seems to follow almost the same pathway as that of the cyclic trimers fully substituted with amino acid esters.[21]

Conclusion

The initial nucleophilic substitution of hexachlorocylotriphosphazene with APEG gave predominantly the cis-nongeminal isomer at low reaction temperature, but a large amount of other cis- and trans-isomers was also produced at room temperature. Heterogeneously substituted thermosensitive trimers, N_3P_3(hydrophilic group)$_x$hydrophobic group)$_{6-x}$, may be synthesized by stereocontrolled partial substitution of $N_3P_3Cl_6$ with low molecular APEG followed by the aminolysis of the remained chloride functions with AAE. The cis-nongeminal products $N_3P_3(APEG)_3(AAE)_3$ could be obtained as highly pure compounds by CP separation, and these trimers exhibited different thermosensitive properties from those of the conventional thermosensitive polymers. The phase transition rate of the present thermosensitive trimers was found to be very fast and responsive even in dilute concentrations. Therefore, some of these thermosensitive trimers appear to be highly promising for application to local drug delivery by using body temperature.

Acknowledgment. This research was financially supported by the Ministry of Science and Technology in Korea. J.-I. Jin expresses his thanks to the Center of Electro- and Photo-Responsive Molecules, Korea University, for support.

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MA010648B