Self-assembly of long-lived *cis*-azobenzenes into crystalline nanoparticles

Mina R. Han,*[a] Daisuke Hashizume[b] and Masahiko Hara[a]

Received (in Montpellier, France) 15th June 2007, Accepted 11th July 2007
First published as an Advance Article on the web 24th July 2007
DOI: 10.1039/b709092g

The development of functional nanostructured materials for optoelectronic and biological applications requires practical approaches to the simple synthesis of specific structures and the long-term stability of optical properties. Here we demonstrate that simple UV light irradiation of a new type of *ortho*-alkylated azobenzene (1-[(*E*)-2-*sec*-butyl-4-(4′-ethoxy-3,5-diethylbiphenyl-4-yldiazenyl)phenoxy]hexadecane, **1**) generates an unusually long-lived *cis*-azobenzene and the self-assembly of the long-lived *cis*-azobenzene drives the formation of strongly fluorescent and so far unknown *cis*-azobenzene nanocrystals of approximately 1–7 nm in size. TEM (transmission electron microscopy) experiments exhibit the characteristic lattice fringe of 2.3 Å spacing from each nanocrystal. Interestingly, because thermal *cis*-to-*trans* back isomerization of **1** possessing bulky substituents at *ortho* positions is significantly restrained, the self-assembled *cis*-azobenzene nanocrystals show highly stable absorbance and fluorescence efficiency for more than 6 months.

Introduction

Advances regarding the synthesis and characterization of fluorescent inorganic nanocrystals such as semiconductors and metal clusters have demonstrated that control of their size, shape, and crystal structure determines their characteristic optical properties.[1–4] Surface modification of such inorganic nanocrystals with specific organic functional groups or biological molecules opens up significant opportunities for their widespread optoelectronic and biological applications. Nevertheless, together with laborious synthetic processes, efforts to modify the surface chemical functionality of such inorganic nanocrystals have often led to stability problems such as obvious changes in the absorption spectrum and fluorescence efficiency.[5] An approach to overcoming these shortcomings of inorganic nanocrystals is to develop new-type fluorescent organic materials. As an example, the photochromic azobenzene molecule, which has been widely used due to its structural change between *trans*- and *cis*-forms and its ease of chemical modification, assembles spontaneously into fluorescent spherical aggregates on UV light irradiation.[6] However, because in general azobenzene derivatives undergo facile *cis*-to-*trans* thermal back isomerization on the time scale of minutes to hours, significant changes in the absorption and fluorescence spectra are observed with time, and therefore, fluorescent azobenzene aggregates composed of only one component of *cis*-azobenzenes could not be isolated and characterized. Accordingly, the design of long-lived *cis*-azobenzenes is necessary to get insight into the details of *cis*-azobenzene-based aggregates and to understand the mechanism of the self-assembly of *cis*-azobenzenes.

Here we show that UV light irradiation of a new type of *ortho*-alkylated azobenzene generates highly stable *cis*-azobenzene at ambient temperature and that the self-assembly of the long-lived *cis*-azobenzene drives the formation of unprecedented, strongly fluorescent *cis*-azobenzene nanocrystals for the first time (Fig. 1). The *cis*-azobenzenes closely packed in the crystalline states are unusually long-lived in solution as well as even in the solid state for more than 1 month, which results in highly stable optical properties. We anticipate that the ease with which azobenzene-based organic nanocrystals can be produced, and the advantage of maintaining stable optical properties and nanostructures over a long period, make photochromic azobenzene molecules more attractive for new optical and optoelectronic materials.

Experimental

A detailed synthetic procedure for **1** has been described previously, and **1** was characterized by ^{1}H NMR, elemental analysis, and X-ray crystal structure analysis.[7] Freshly distilled dichloromethane under nitrogen atmosphere was used to dissolve the azobenzene compound so as to remove extra oxygen. After a 1 min nitrogen purge, a screw-cap quartz cuvette containing azobenzene solution was sealed with Parafilm®. Azobenzene solutions were irradiated with UV light (365 nm, 3–4 mW cm^{-2}, Mineralight® lamp, Model UVGL-25, UVP, Upland, CA 91786, USA) at ambient temperature.

Absorption and fluorescence spectra were obtained using a Shimadzu UV-3100PC UV-VIS-NIR scanning spectrophotometer and a JASCO FP-6500 spectrofluorometer, respectively. ^{1}H NMR and attenuated total reflection Fourier transform infrared spectroscopy (ATR/FT-IR) spectra were recorded on a JEOL JNM-EX270 (270 MHz) and a Nicolet OMNI-Sampler (Avatar 320-FT-IR) spectrometer, respectively. The FE-SEM (field-emission scanning electron microscopy) was recorded on a Hitachi S-5200, after putting one drop of the UV-exposed solution on a clean glass substrate

---

[a] *Local Spatio-Temporal Functions Laboratory, Frontier Research System, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan. E-mail: minahjp@riken.jp; Fax: +81 48 462 4695; Tel: +81 48 462 1111*
[b] *Molecular Characterization Team, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*

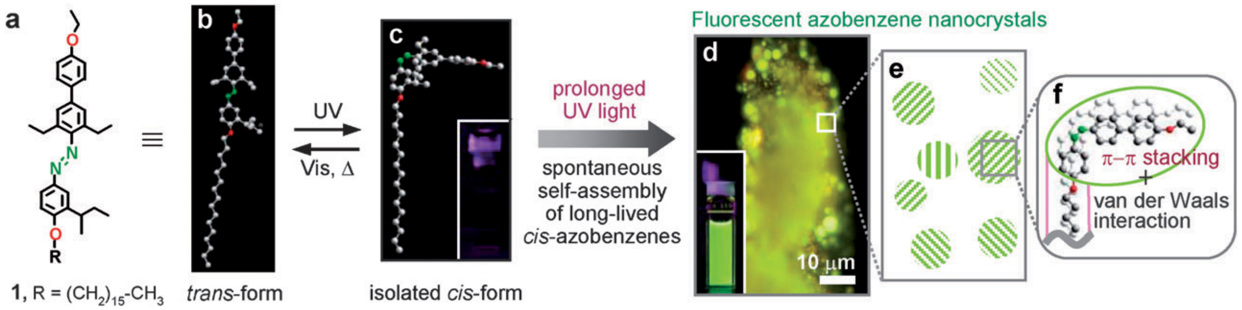

Fig. 1 (a) Molecular structure of **1**. (b) X-ray crystal structure of **1**.[7] Hydrogen atoms are omitted for clarity (C, gray; O, red; N, green). (c) Isolated (nonaggregated) *cis*-form. The optimized structure of the *cis*-isomer in the gas phase was obtained using the AM1 method as implemented in the HyperChem 7.5 program (Hypercube, Inc., Gainsville, FL). Inset: No fluorescence was observed from the *trans*-rich and isolated *cis*-rich states. (d) Fluorescence microscopy image of **1** observed after placing a few drops of the solution on a quartz substrate and drying at ambient temperature. Inset: Greenish-yellow fluorescence from the UV-light irradiated solution under 365-nm light irradiation. (e) and (f) Schematic representation of the self-assembly of *cis*-azobenzenes into fluorescent nanocrystals. Alkyl chains attached to azobenzene units in (f) are omitted for clarity.

and coating it with platinum about 2 nm thick using the Hitachi E-1030 ion-sputter. The TEM (transmission electron microscopy) was performed at 120 kV using a JEOL JEM-2100F/SP. The sample was prepared by placing a few drops of the UV-exposed solution onto carbon-coated grids as well as silicon oxide-coated grids, and dried at room temperature for more than 2 hours. A fluorescence microscopic image of the air-dried sample was obtained using an Olympus BX51WI fluorescence microscope equipped with a Nikon Coolpix digital camera (MDC Lens 0.82–0.29X, Nikon Japan), after putting a few drops of **1** solution on a clean quartz substrate.

Results and discussion

The experimental verification of our design strategy for obtaining long-lived *cis*-azobenzene is based on *ortho*-alkylated azobenzene, 1-[(*E*)-2-*sec*-butyl-4-(4′-ethoxy-3,5-diethylbiphenyl-4-yldiazenyl)phenoxy]hexadecane (**1**). Bunce and coworkers reported that azobenzene compounds possessing bulky substituents at *ortho* positions can form stable *cis*-isomers at ambient temperature,[8,9] because bulky substituents prevent large-scale distortion (such as rotation and/or inversion) of the azo group for thermal *cis*-to-*trans* isomerization.

Azobenzene compound was dissolved in freshly distilled dichloromethane at a concentration of 4×10^{-5} M for absorption and fluorescence spectroscopic studies. UV light irradiation (365 nm) of the nonfluorescent initial solution for 3 min results in a sufficient *cis*-rich stationary state, accompanying both a prominent decrease in the π–π* absorption band at around 350 nm and a shape change in the n–π* absorption band near 450 nm (Fig. 2a). Giving a product obviously different from the isolated *cis*-rich state, prolonged UV light irradiation leads to drastic changes in both π–π* and n–π* absorption bands together with striking fluorescence enhancement at 529 nm (Figs. 1d and 2b). The fluorescence quantum yield for the UV-exposed azobenzene solution was 12.5 ± 1.4% with fluorescein dissolved in 0.1 N sodium hydroxide as a reference.[10] Surprisingly, no further changes in absorption and fluorescence spectra are observed in response to long-term dark incubation for 1 month and even for more than 6 months, strongly suggesting that no thermal *cis*-to-*trans* back isomerization takes place in dilute solution. Considering that in general *cis*-azobenzene reverts fully to

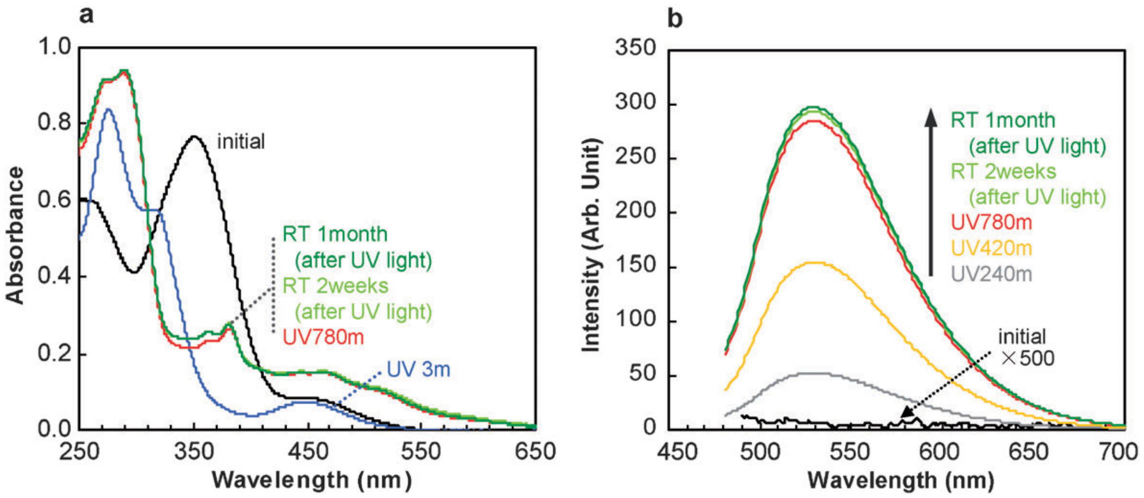

Fig. 2 Changes in (a) UV–Vis absorption and (b) fluorescence spectra (365 nm excitation) of **1** (4×10^{-5} M).

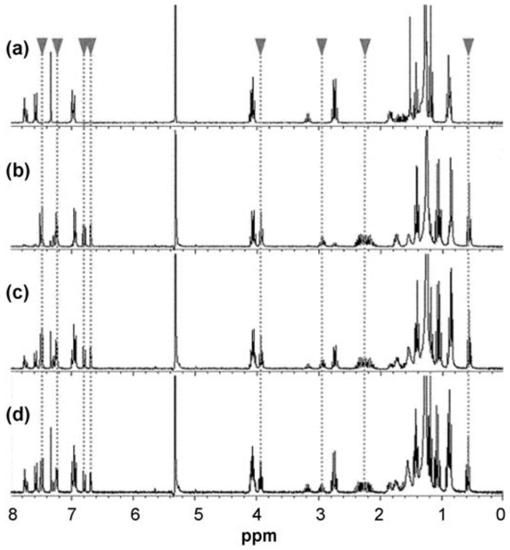

Fig. 3 ^{1}H NMR spectra of **1** in CD_2Cl_2 (5×10^{-3} M). (a) Initial (all *trans*) state. (b) After UV light irradiation for 20 hours (*cis*-rich state). (c) After dark incubation for 2 weeks after UV light irradiation (*cis*-isomer fraction: 67%). (d) After dark incubation for 1 month (*cis*-isomer fraction: 55%).

trans-azobenzene on the time scale of minutes to hours,[6,9] the *cis*-form of *ortho*-alkylated azobenzene **1** is extraordinarily long-lived. Further evidence for the existence of long-lived *cis*-azobenzene in the UV-exposed solution was confirmed by NMR experiments (5×10^{-3} M). More than 50% of *cis*-isomer still remained after dark incubation of the UV-exposed solution for 1 month, as shown in Fig. 3.

Aggregation behavior of long-lived *cis*-azobenzenes and the resulting fluorescence enhancement by UV light irradiation were investigated by using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and fluorescence microscopy. SEM images show the existence of not only small spherical aggregates with diameters of less than 20 nm but also larger-sized clusters ranging from several tens of nanometers to hundreds of nanometers in size (Fig. 4a and b). TEM images of the sample clearly exhibit nanometer-scale aggregates of approximately 1–7 nm in size (Fig. 4c–e). Brighter contrast between darker regions corresponding to the azobenzene aggregates implies that each aggregate is randomly dispersed. Detailed TEM characterization exhibits the crystalline nature of the *cis*-azobenzene aggregates. The lattice fringe of 2.3 Å spacing in the high magnification image is clearly seen from each separate aggregate on carbon-coated grids (Fig. 4d) as well as silicon oxide-coated grids (Fig. 4e), which may be closely associated with a characteristic spacing of lattice planes of the *cis*-azobenzene units assembling into nanocrystals. Nevertheless, the exact structural characterization of the *cis*-azobenzene-based nanocrystal is still unclear. Further investigations are underway to clarify the crystal structure of the azobenzene aggregates. In addition to SEM and TEM experiments, a fluorescence microscopy image of the air-dried sample displays strong greenish-yellow fluorescence virtually identical to the fluorescence color from the solution (Fig. 1d). The results described above strongly verify that the marvelous absorption and fluorescence spectral features originate from the self-assembly of *cis*-azobenzenes into fluorescent crystalline aggregates, as observed from unique organic molecules exhibiting aggregation-induced fluorescence enhancement.[6,11,12]

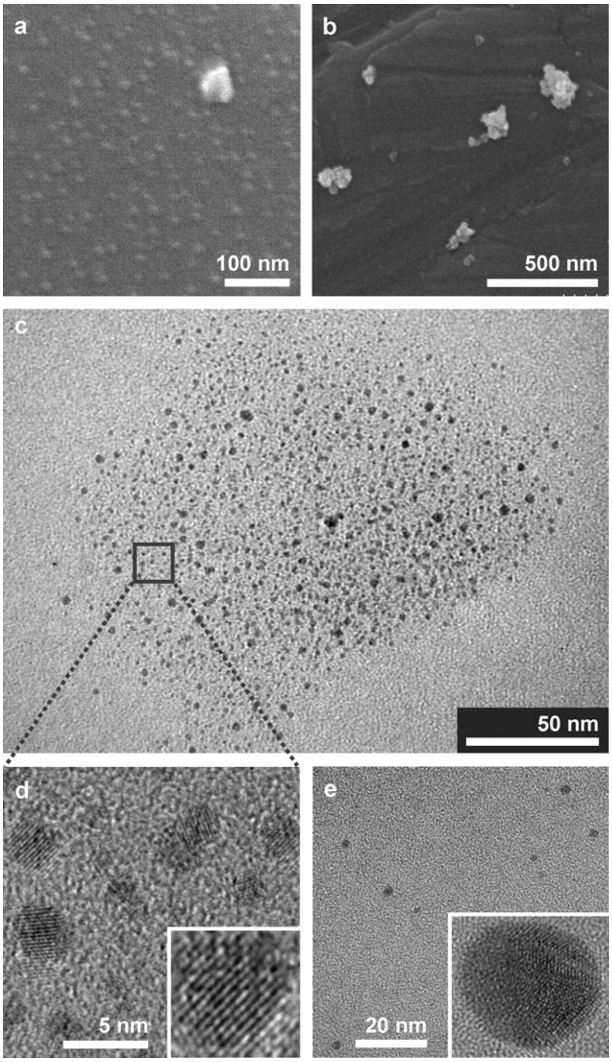

Fig. 4 SEM and TEM images of *cis*-azobenzene nanocrystals from the UV-exposed solution (4×10^{-5} M). SEM images exhibit the presence of (a) small spherical aggregates of less than 20 nm in diameter as well as (b) larger-sized clusters formed by the coalescence of small aggregates. TEM images of the *cis*-azobenzene nanocrystals were taken on (c, d) carbon-coated grids and (e) silicon oxide-coated grids. Insets in d and e are high resolution images that clearly show the lattice fringe of 2.3 Å spacing from each nanocrystal.

To understand how the spontaneous self-assembly of long-lived *cis*-azobenzenes into highly fluorescent nanocrystals occurs, an X-ray diffraction (XRD) study was performed. As shown in Fig. 5, XRD data before and after UV light irradiation are completely different, indicating that UV light irradiation leads to significant changes in crystal structures between initial *trans*-azobenzene crystals and *cis*-azobenzene-based nanocrystals. In particular, two notable peaks, 3.82 Å and 4.48 Å, seem to hint at how *cis*-azobenzenes assemble into nanocrystals. First, the strongest peak of 3.82 Å appearing in the spectrum after UV light irradiation is likely to arise from

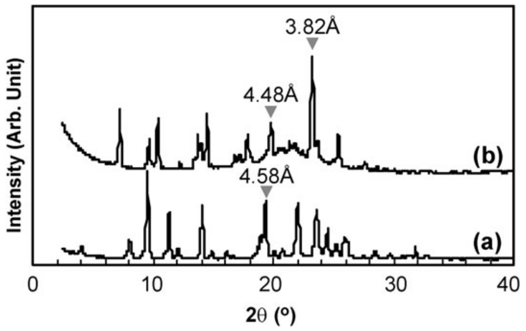

Fig. 5 X-ray diffraction patterns of **1** (a) before and (b) after UV light irradiation.

the π–π stacked structures between the azobenzene aromatic rings, as observed from graphite structures,[13] diphenylbutadiene,[14] hexa-peri-hexabenzocoronene,[15] and so on.[16] Further evidence for the π–π stacking between *cis*-azobenzenes can be provided by absorption spectral data. Obviously different from the absorption spectrum of nonaggregated *cis*-azobenzene (corresponding to after UV light irradiation for 3 min in Fig. 2a), two peaks at 364 nm and 382 nm as well as strong *n*–π* absorption bands appear in the spectrum of prolonged UV light-irradiated solution. This unique spectral feature is related to the closely packed structure of long-lived *cis*-azobenzene aromatic rings. Additionally, attenuated total reflection Fourier transform infrared spectroscopy (ATR/FT-IR) data show that the absorption bands attributable to the azobenzene aromatic unit at 1595 ($\nu_{\mathrm{ring\ C\text{–}C}}$), 1515 ($\nu_{\mathrm{ring\ C\text{–}C}}$), 1244 ($\nu_{\mathrm{Ph\text{–}O\text{–}C}}$), 1176 ($\nu_{\mathrm{Ph\text{–}N}}$), 1045 ($\nu_{\mathrm{Ph\text{–}O\text{–}C}}$) cm^{-1} are shifted to higher frequencies and become weaker relative to those of the initial *trans*-rich state (Fig. 6), which is due to typical *trans*-to-*cis* photoisomerization.[17–19] These XRD, UV–Vis, and IR spectral changes suggest that the strong π–π stacking between long-lived *cis*-azobenzene aromatic units is one of the important factors elucidating how *cis*-azobenzenes spontaneously assemble into crystalline aggregates in solution.

Moreover, the unusual fluorescence enhancement in *cis*-azobenzene nanocrystals can be explained in terms of π–π stacking of *cis*-azobenzenes with more planar structure, even though isolated and nonfluorescent *cis*-azobenzenes generated in the first stage of the *trans*-to-*cis* photoisomerization process deviate from a planar conformation. For planar *cis*-azobenzenes that are locked against rotation/inversion for thermal *cis*-to-*trans* isomerization by bulky alkyl groups at *ortho* positions, a conventional selection rule for the radiationless decay may be forbidden, as suggested by Bisle and Rau.[20] That is, a more planar conformation of *cis*-azobenzene might play a crucial role in stimulating the unique self-assembly into fluorescent crystalline aggregates.

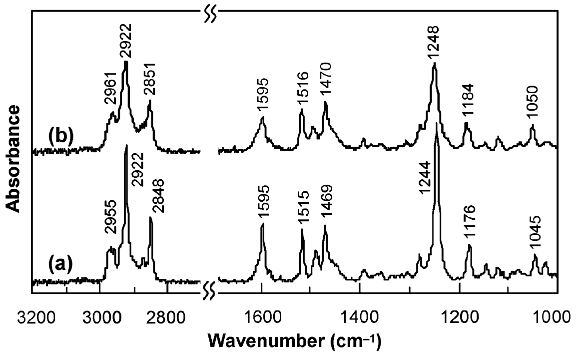

Fig. 6 ATR/FT-IR spectra of **1** (a) before and (b) after UV light irradiation.

The second and significant peaks at 4.58 Å and 4.48 Å from XRD data taken before and after UV light irradiation, respectively, are assignable to the stacking distances of the well-aligned long alkyl chains.[21,22] Furthermore, the characteristic asymmetric (CH_3 and CH_2) and symmetric (CH_2) stretchings at 2961 cm^{-1}, 2922 cm^{-1}, and 2851 cm^{-1}, respectively, appear in the IR spectra taken before and after UV light irradiation (Fig. 6),[23,24] indicating that a spatially ordered arrangement of long alkyl chains still remains to a high extent in the *cis*-azobenzene nanocrystals. From the data described above, it is concluded that a significant interplay of strong π–π stacking between *cis*-azobenzene aromatic rings and van der Waals interactions between long alkyl chains facilitates the packing of *cis*-azobenzene molecules to achieve highly fluorescent crystalline aggregates.

Conclusions

We have demonstrated that upon simple UV light irradiation a new type of long-lived *cis*-azobenzenes self-assembles into novel *cis*-azobenzene nanocrystals. The *cis*-azobenzene-based nanocrystals exhibit strong greenish-yellow fluorescence in solution as well as in the solid state and highly stable optical properties such as absorbance spectrum and fluorescence efficiency. The present study suggests the possibility of overcoming the drawbacks of fluorescent inorganic nanocrystals such as synthesis and stability problems and the findings will be applicable to a wide variety of biological probes, sensors, and light-emitting diodes.

Acknowledgements

We are grateful to Ms. Emi Muto and Dr Shinya Onoue for their assistance with TEM (transmission electron microscope) experiments and Ms. Masami Tanaka for technical assistance.

References

1 F. X. Redl, K.-S. Cho, C. B. Murray and S. O'Brien, *Nature*, 2003, **423**, 968.
2 H. Cölfen and S. Mann, *Angew. Chem., Int. Ed.*, 2003, **42**, 2350.
3 S. Coe, W. Woo, M. Bawendi and V. Bulović, *Nature*, 2002, **420**, 800.
4 L. Manna, E. C. Scher and A. P. Alivisatos, *J. Am. Chem. Soc.*, 2000, **122**, 12700.
5 J. Aldana, Y. A. Wang and X. Peng, *J. Am. Chem. Soc.*, 2001, **123**, 8844.
6 (*a*) M. R. Han and M. Hara, *New J. Chem.*, 2006, **30**, 223; (*b*) M. R. Han, Y. Hirayama and M. Hara, *Chem. Mater.*, 2006, **18**, 2784; (*c*) M. Han and M. Hara, *J. Am. Chem. Soc.*, 2005, **127**, 10951.
7 M. R. Han, D. Hashizume and M. Hara, *Acta Crystallogr., Sect. E: Struct. Rep. Online*, 2006, **62**, o3001.
8 (*a*) N. J. Bunce, G. Ferguson, C. L. Forber and G. J. Stachnyk, *J. Org. Chem.*, 1987, **52**, 394; (*b*) C. L. Forber, E. C. Kelusky, N. J. Bunce and M. C. Zerner, *J. Am. Chem. Soc.*, 1985, **107**, 5884.

9 *Photoreactive Organic Thin Films*, ed. Z. Sekkat and W. Knoll, Academic Press, Elsevier Science, USA, 2002.

10 W. R. Dawson and M. W. Windsor, *J. Phys. Chem.*, 1968, **72**, 3251.

11 (*a*) B.-K. An, S.-K. Kwon, S.-D. Jung and S. Y. Park, *J. Am. Chem. Soc.*, 2002, **124**, 14410; (*b*) B.-K. An, D.-S. Lee, J.-S. Lee, S.-Y. Park, H.-S. Song and S. Y. Park, *J. Am. Chem. Soc.*, 2004, **126**, 10232.

12 (*a*) J. Luo, Z. Xie, J. W. Y. Lam, L. Cheng, H. Chen, C. Qiu, H. S. Kwok, X. Zhan, Y. Liu, D. Zhu and B. Z. Tang, *Chem. Commun.*, 2001, 1740; (*b*) H. Tong, Y. Hong, Y. Dong, Y. Ren, M. Häussler, J. W. Y. Lam, K. S. Wong and B. Z. Tang, *J. Phys. Chem. B*, 2007, **111**, 2000.

13 S. Iijima and T. Ichibashi, *Nature*, 1993, **363**, 603.

14 R. Davis, N. P. Rath and S. Das, *Chem. Commun.*, 2004, 74.

15 J. P. Hill, W. Jin, A. Kosaka, T. Fukushima, H. Ichihara, T. Shimomura, K. Ito, T. Hashizume, N. Ishii and T. Aida, *Science*, 2004, **304**, 1481.

16 E. Mena-Osteritz, *Adv. Mater.*, 2002, **14**, 609.

17 N. Tillman, A. Ulman, J. S. Schildkraut and T. L. Prenner, *J. Am. Chem. Soc.*, 1988, **110**, 6136.

18 T. Sato, Y. Ozaki and K. Iriyama, *Langmuir*, 1994, **10**, 2363.

19 (*a*) M. Han, S. Morino and K. Ichimura, *Macromolecules*, 2000, **33**, 6360; (*b*) M. Han and K. Ichimura, *Macromolecules*, 2001, **34**, 90.

20 H. Bisle and H. Rau, *Chem. Phys. Lett.*, 1975, **31**, 264.

21 A. N. Parikh, S. D. Gillmor, J. D. Beers, K. M. Beardmore, R. W. Cutts and B. I. Swanson, *J. Phys. Chem. B*, 1999, **103**, 2850.

22 R. Lim, J. Li, S. F. Y. Li, Z. Feng and S. Valiyaveettil, *Langmuir*, 2000, **16**, 7023.

23 S. Chang, I. Chao and Y. Tao, *J. Am. Chem. Soc.*, 1994, **116**, 6792.

24 T. Seki, K. Ichimura, R. Fukuda, T. Tanigaki and T. Tamaki, *Macromolecules*, 1996, **29**, 892.