Tetrahedron Letters 56 (2015) 824–827

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Fluorinated perylene diimides: synthesis, electrochemical–photophysical properties, and cellular imaging

Jia Wang [a], Shilong Zhong [a], Wenfeng Duan [a], Baoxiang Gao [a,b,*]

[a] College of Chemistry and Environmental Science, Hebei University, Baoding 071002, PR China
[b] Key Laboratory of Medicinal Chemistry and Molecular Diagnosis, Ministry of Education, Baoding 071002, PR China

ARTICLE INFO

Article history:
Received 3 October 2014
Revised 11 December 2014
Accepted 19 December 2014
Available online 31 December 2014

Keywords:
Perylene diimides
Fluorination
Electrochemical properties
Photophysical properties
Cellular imaging

ABSTRACT

We report the synthesis and properties of perylene diimides with fluorinated substituents on the bay (**BFPDI**s). These **BFPDI**s exhibit good water solubility, high extinction coefficients, and high fluorescence quantum yields. Furthermore, these **BFPDI**s are used as probes in cellular imaging.

Introduction

Perylene diimides (**PDI**s) represent a class of organic chromophores with photochemical stabilities, high extinction coefficients, and high quantum yields.[1,2] **PDI**s are key chromophores for high-tech applications, such as organic photovoltaics,[3] organic field-effect transistors,[4] biolabeling,[5] sensors,[6] single molecular spectroscopy,[7] and supramolecular assemblies.[8] Stability, chemical robustness, and ease of preparation are among a few of the necessary characteristics for organic chromophores used in these fields.[9] Currently available synthetic methods allow the preparation of stable chromophores with increasingly negative reduction potentials.[10]

Fluorine is the strongest element with electron affinity and a small atom that can be introduced onto molecules with minimal effect on steric hindrance. Highly fluorinated materials display a variety of interesting properties, such as thermal and chemical stability, low surface energy, and high resistance to oxidation.[11] Swager and co-workers have recently reported two highly fluorinated poly(*p*-phenylene ethynelene)s with outstanding fluorescence quantum yields in solution and in thin films.[12] Furthermore, fluorous polymers are also biocompatible. Zhang et al. reported a highly fluorescent fluorinated semiconducting polymer dot that is eight times brighter in cell-labeling applications than its non-fluorinated counterpart.[13] These successful applications point to the potential of fluorinated chromophores in biological applications.

Our group aims to develop photochemically stable and biocompatible perylene dyes with high fluorescence in aqueous solutions for bioimaging.[14] In this study, we report the synthesis of perylene diimides with fluorinated substituents on the bay (**BFPDI**s), as well as their electrochemical–photophysical properties and applications for cellular imaging.

Results and discussion

Chart 1 shows the chemical structures of **BPPDI** and **BFPDI**s, which were efficiently synthesized by the stepwise synthetic protocol illustrated in Scheme S1. **BPPDI** without fluorinated substituents on the bay was used as a reference compound to study the mechanisms by which fluorinated substituents affect the properties of **PDI**s. Dibromo-perylene tetracarboxylic dianhydride[15] and 2,5,8,12,15,18-hexaoxa-10-nonadecanamine[16] were prepared according to the literature procedures. The reaction of 2,5,8,12,15,18-hexaoxa-10-nonadecanamine **1** with Cbz-protected L-aspartic acid yielded compound **2**. The Cbz group was removed by catalytic hydrogenation with Pd/C to obtain compound **3**. Compound **5** was obtained via a coupling reaction between dibromo-perylene tetracarboxylic dianhydride and compound **3**. **BPPDI** and **BFPDI**s compounds were prepared by Suzuki coupling with compound **5** and the corresponding phenylboronic acid, followed

* Corresponding author. Tel.: +86 312 3382004; fax: +86 312 5079317.
E-mail address: bxgao@hbu.edu.cn (B. Gao).

http://dx.doi.org/10.1016/j.tetlet.2014.12.112
0040-4039/

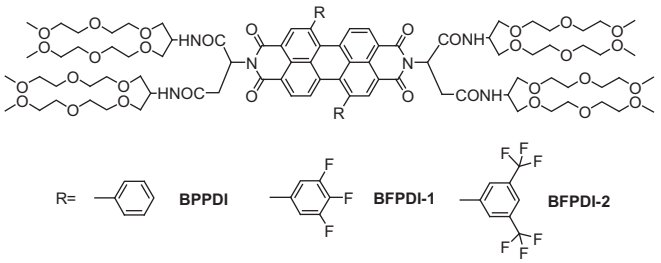

Chart 1. Chemical structures of **BPPDI** and **BFPDI**s.

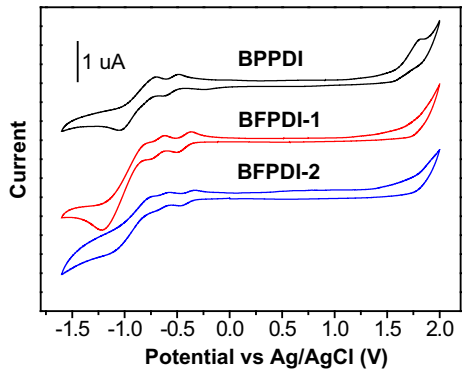

Figure 1. Cyclic voltammograms of compounds **BPPDI** and **BFPDI**s in dichloromethane at a scan rate of 0.1 V/s.

by purification through column chromatography on silica gel. These new perylene diimides were fully characterized by ^{1}H NMR spectroscopy, ^{13}C NMR spectroscopy and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF-MS). Details of the synthetic route and all spectroscopies are provided in the Supporting information.

The electrochemical properties of **BPPDI** and **BFPDI**s in dichloromethane were studied in a three-electrode electrochemical cell using Bu_4NPF_6 (0.1 M) and Ag/AgCl as the electrolyte and the reference electrode, respectively. The CV curves are shown in Figure 1. **BPPDI** revealed one irreversible oxidation potential at 1.69 V versus Ag/AgCl and one reversible reduction potential at −0.55 V versus Ag/AgCl. After installing the fluorinated substituents on the bay of **PDI**s, the first reduction potentials of **BFPDI**s (−0.40 V for **BFPDI-1**, −0.38 V for **BFPDI-2**) were shifted by approximately 150 mV toward more positive potentials compared with that of **BPPDI**. These **BFPDI**s showed an increase in the first reduction potential, indicating that the electron-accepting power increased in strength. By contrast, the CV scan for the **BFPDI**s with anodic scanning from 0 to 2 V showed no peaks, suggesting that the electron-donating property of the **BFPDI**s became weak. The lowest unoccupied molecular orbital (LUMO) levels were estimated from the onset of the first reduction potentials.[17] LUMO levels of the three compounds were calculated as −3.85 eV, −4.00 eV, and −4.02 eV, respectively. The decreased LUMO levels are ascribed to the electron-deficient fluorinated substituents on the bay of perylene diimides.

The optical properties of **BPPDI** and **BFPDI**s in water were studied and compared with those in toluene. In the hydrophobic toluene solvent, the absorption maximum of **BPPDI** appeared at 551 nm, along with higher vibronic transitions located at 518 nm (Fig. 2A). With fluorinated benzene on the bay, the maximum absorption of the **BFPDI**s was blue-shifted to 542 nm for **BFPDI-1** and 539 nm for **BFPDI-2**. Interestingly, the extinction coefficients of these fluorinated **PDI**s were higher than that of the non-fluorinated counterpart. The photoluminescence (PL) peak of **BPPDI** in toluene solution appeared at 606 nm. With fluorinated substitu-

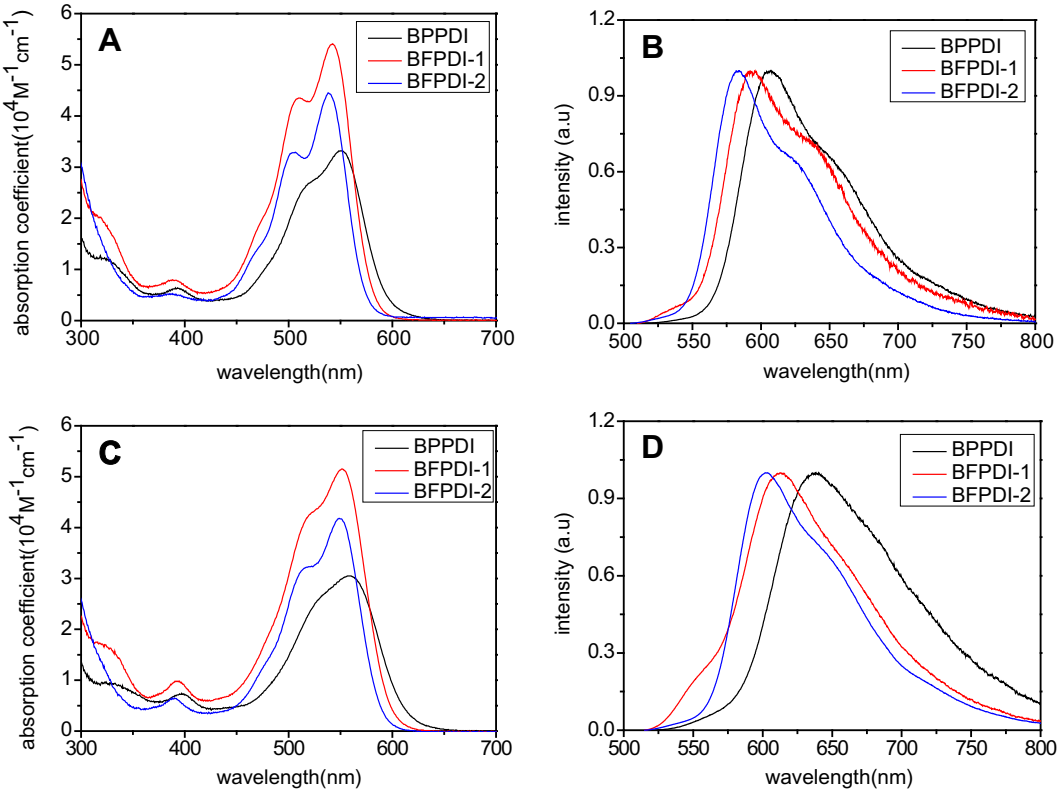

Figure 2. UV–vis absorption at 1.0×10^{-4} M and PL spectroscopy at 1.0×10^{-5} M of **PDI**s in toluene solution (A, B) and aqueous solution (C, D).

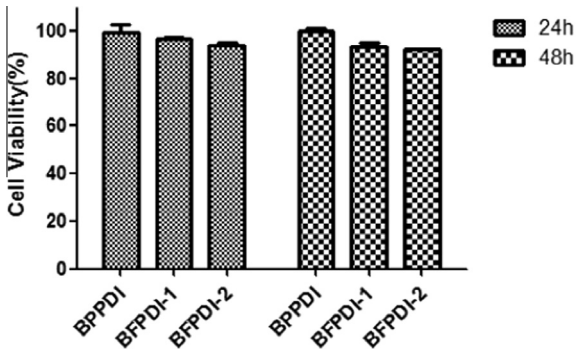

Figure 3. In vitro viability of HeLa cells treated with **BPPDI** and **BFPDI**s solutions at 2.5×10^{-5} mol L^{-1} for 24 and 48 h, respectively.

ents, the PL peaks of these **PDI**s also were blue-shifted to 593 nm for **BFPDI-1** and 583 nm for **BFPDI-2**. Furthermore, these **PDI**s showed similar quantum yields of 65–74%. The relatively high quantum yields suggest the minimal aggregation of the **PDI**s in toluene solution.[18]

These **PDI**s are highly soluble in water. In aqueous solution, the absorption maximum of these **PDI**s was red-shifted to 557 nm for **BPPDI**, 550 nm for **BFPDI-1**, and 548 nm for **BFPDI-2**, compared with those in toluene solution. The PL peaks of these **PDI**s appeared at 637 nm for **BPPDI**, 613 nm for **BFPDI-1**, and 603 nm for **BFPDI-2**. **BPPDI** revealed a low fluorescence quantum yield of 15% in aqueous solution. With fluorinated substituents on the bay, the fluorescence quantum yields of these **PDI**s were improved to 28% for **BFPDI-1** and 36% for **BFPDI-2**. The continuously enhanced quantum yields may be attributed to the strong hydrophobic properties of F atoms together with F-F and/or F-H interactions, which minimized the aggregation-induced quenching.[13]

The cytocompatibility or cytotoxicity of **BPPDI** and **BFPDI**s must be assessed to demonstrate their potential utility in cellular imaging. The biocompatibility of **BPPDI** and **BFPDI**s was evaluated in HeLa cells using the **MTT** cell-viability assay. Figure 3 summarizes the viability of HeLa cells after being cultured with **BPPDI** and **BFPDI**s solutions at a concentration of 2.5×10^{-5} mol L^{-1} for 24 and 48 h. These compounds showed very low cytotoxicity (over 90% viability) after 48 h of incubation. This result indicates that the introduction of fluorine has almost no effect on the biocompatibility of **BFPDI**s. This bodes well for the utility of this fluorinated fluorescent probe, particularly in live cell imaging applications and the research of the biological active substances.

Live cell imaging based on these **PDI**s was investigated using confocal laser scanning microscopy (CLSM). After being incubated with these **PDI** solutions (1.0×10^{-5} mol L^{-1}) for 45 min, the cells were washed three times with PBS buffer. The excitation wavelength was fixed at 488 nm, and fluorescent signals were collected from 535 nm to 635 nm for **BFPDI-1** and 560–660 nm for **BPPDI** and **BFPDI-2**. Figure 4D shows the confocal microcopy images of HeLa cells incubated with **BPPDI**, where weak fluorescence was detected. The low brightness of images is attributed to the low fluorescence quantum yield of **BPPDI**. However, the fluorescence from the cells stained by **BFPDI-1** was enhanced (Fig. 4E). Figure 4F shows strong fluorescence from the cells stained by **BFPDI-2**. This finding reveals that these **BFPDI**s can efficiently accumulate in live cells and perform cellular imaging. Changes in the fluorescence images of HeLa cells stained by **BPPDI** and **BFPDI**s under continuous 488 nm laser (10% of laser intensity) irradiation were monitored to evaluate the photostability of **BPPDI** and **BFPDI**s in cells. After irradiation for 1 min, the intensity of the fluorescence images remained almost unchanged (Fig. S1 in the Supporting information). These results prove the relatively high photostability of **BPPDI** and **BFPDI**s in harsh physiological environment. Thus, these

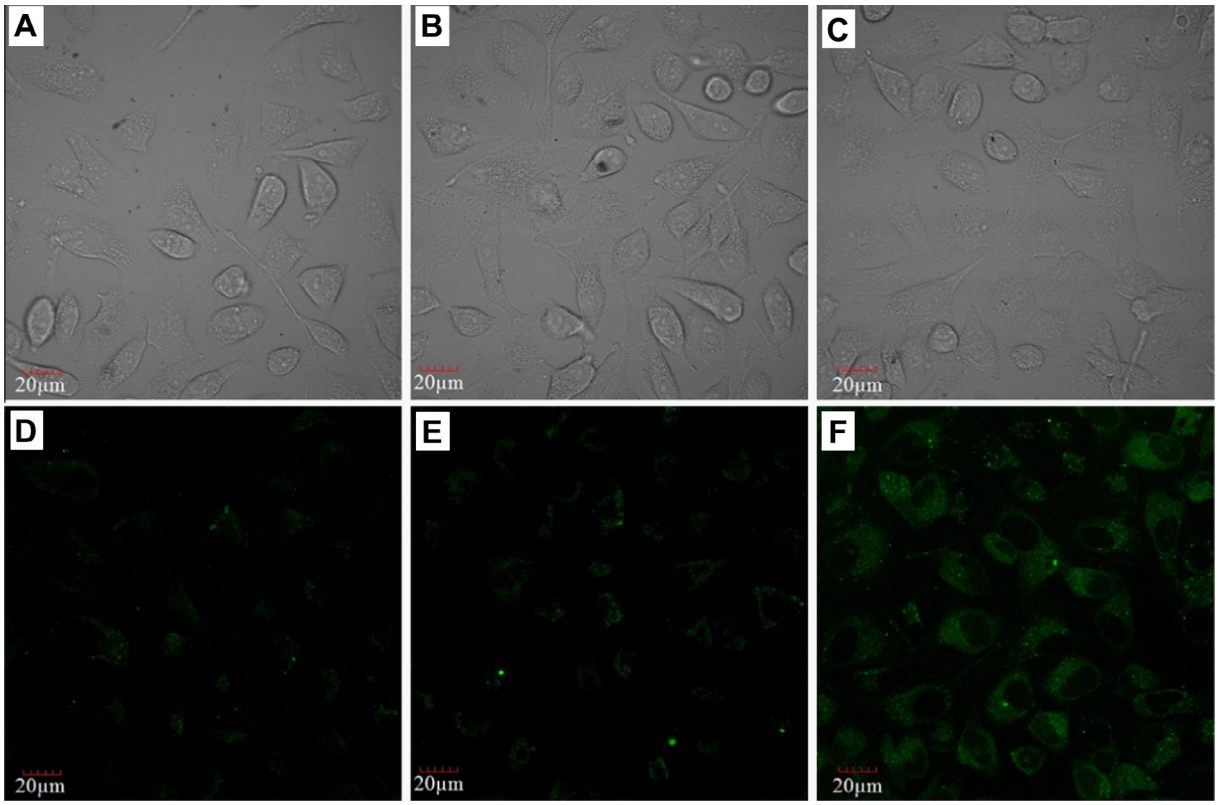

Figure 4. The bright-field images of Hela cells stained by **BPPDI** (A), **BFPDI-1** (B), and **BFPDI-2** (C) and the confocal fluorescence images of Hela cells stained by **BPPDI** (D), **BFPDI-1** (E), and **BFPDI-2** (F).

highly fluorescent perylene diimides with fluorine atoms have potential applications as probes for molecular imaging.

Conclusions

Fluorinated perylene diimides were synthesized by installing fluorinated substituents on the bay of perylene diimides. With fluorinated substituents, these perylene diimides showed increased first reduction potential, low cytotoxicity, good water solubility and photostability, and high extinction coefficients and fluorescence quantum yields in aqueous solution. Furthermore, these fluorinated perylene diimides can efficiently accumulate in live cells and perform cellular imaging.

Acknowledgements

This work was supported by the Program for Distinguished Young Scholar of Hebei Province China (B2012201031), the National Natural Science Foundation China (21274036), the Program for New Century Excellent Talents in University China (NCET-12-0684), the Program for Training Innovative Research Team and Leading talent in Hebei Province University China (LJRC024), the Program for Innovative talent in Hebei Province University China (GCC2014054).

Supplementary data

Supplementary data (detailed synthetic procedures and characterization, general procedures, and supporting spectroscopic) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.12.112.

References and notes

1. (a) Demmig, S.; Langhals, H. *Chem. Ber.* **1988**, *121*, 225–230; (b) Herbst, W.; Hunger, K. *Industrial Organic Pigments*, 2nd completely revised ed.; Wiley-VCH: Weinheim, 1997; (c) Zollinger, H. *Color Chemistry*, 3rd ed.; Wiley-VCH: Weinheim, 2003; (d) Würthner, F. *Chem. Commun.* **2004**, 1564–1579.
2. Seybold, G.; Wagenblast, G. *Dyes Pigm.* **1989**, *11*, 303–317.
3. (a) Schmidt-Mende, L.; Fechtenkötter, A.; Müllen, K.; Moons, E.; Friend, R. H.; MacKenzie, J. D. *Science* **2001**, *293*, 1119–1122; (b) Neuteboom, E. E.; Meskers, S. C. J.; van Hal, P. A.; Van Duren, J. K. J.; Meijer, E. W.; Janssen, R. A. J.; Dupin, H.; Pourtois, G.; Cornil, J.; Lazzaroni, R.; Brédas, J.-L.; Beljonne, D. *J. Am. Chem. Soc.* **2003**, *125*, 8625–8638.
4. (a) Jones, B. A.; Ahrens, M. J.; Yoon, M. H.; Facchetti, A.; Marks, T. J.; Wasielewski, M. R. *Angew. Chem., Int. Ed.* **2004**, *43*, 6363–6366; (b) Jones, B. A.; Facchetti, A.; Wasielewski, M. R.; Marks, T. J. *J. Am. Chem. Soc.* **2007**, *129*, 15259–15278.
5. (a) Peneva, K.; Mihov, G.; Nolde, F.; Rocha, S.; Hotta, J.; Braeckmans, K.; Hofkens, J.; Uji-i, H.; Herrmann, A.; Müllen, K. *Angew. Chem., Int. Ed.* **2008**, *47*, 3372–3375; (b) Abdalla, M. A.; Bayer, J.; Rädler, J. O.; Müllen, K. *Angew. Chem., Int. Ed.* **2004**, *43*, 3967–3970; (c) Peneva, K.; Mihov, G.; Herrmann, A.; Zarrabi, N.; Börsch, M.; Duncan, T. M.; Müllen, K. *J. Am. Chem. Soc.* **2008**, *130*, 5398–5399.
6. (a) Yan, L. W.; Yang, L.; Lan, J. B.; You, J. S. *Sci. China B* **2009**, *52*, 518–522; (b) Soh, N.; Ariyoshi, T.; Fukaminato, T.; Nakano, K.; Irie, M.; Imato, T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2943–2946; (c) Soh, N.; Ariyoshi, T.; Fukaminato, T.; Nakajima, H.; Nakano, K.; Imato, T. *Org. Biomol. Chem.* **2007**, *5*, 3762–3768; (d) Ito, F.; Ariyoshi, T.; Soh, N.; Kakiuchi, T.; Inoue, T.; Imato, T.; Nagamura, T. *Chem. Lett.* **2008**, *37*, 1202–1203.
7. (a) Göhde, W.; Fischer, U. C.; Fuchs, H.; Tittel, J.; Basché, Th.; Bräuchle, Ch.; Herrmann, A.; Müllen, K. *J. Phys. Chem. A* **1998**, *102*, 9109–9116; (b) Stracke, F.; Blum, C.; Becker, S.; Müllen, K.; Meixner, A. J. *Chem. Phys. Lett.* **2000**, *325*, 196–202; (c) Stracke, F.; Blum, C.; Becker, S.; Müllen, K.; Meixner, A. J. *Chem. Phys.* **2004**, *300*, 153–164; (d) Vallée, R. A. L.; Cotlet, M.; Auweraer, M. V.; Hofkens, J.; Müllen, K.; De Schryver, F. C. *J. Am. Chem. Soc.* **2004**, *126*, 2296–2297; (e) Dill, M.; Baier, M. C.; Mecking, S.; Wöll, D. *Angew. Chem., Int. Ed.* **2013**, *52*, 12435–12438.
8. (a) Würthner, F. *Pure Appl. Chem.* **2006**, *78*, 2341–2349; (b) Chen, Z. J.; Lohr, A.; Saha-Möller, C. R.; Würthner, F. *Chem. Soc. Rev.* **2009**, *38*, 564–584; (c) Chen, L.; Li, C.; Müllen, K. *J. Mater. Chem. C* **2014**, *2*, 1938–1956.
9. Zhan, X.; Facchetti, A.; Barlow, S.; Marks, T. J.; Ratner, M. A.; Wasielewski, M. R.; Marder, S. R. *Adv. Mater.* **2011**, *23*, 268–284.
10. (a) Schmidt, R.; Oh, J. H.; Sun, Y. S.; Deppisch, M.; Krause, A. M.; Radacki, K.; Braunschweig, H.; Könemann, M.; Erk, P.; Bao, Z.; Würthner, F. *J. Am. Chem. Soc.* **2009**, *131*, 6215–6228; (b) Chen, K. Y.; Chow, T. J. *Tetrahedron Lett.* **2010**, *51*, 5959–5963; (c) Roznyatovskiy, V. V.; Gardner, D. M.; Eaton, S. W.; Wasielewski, M. R. *Org. Lett.* **2014**, *16*, 696–699.
11. Kirsch, P. *Modern Fluoroorganic Chemistry*; Wiley-VCH: Weinheim, 2004.
12. Lim, J.; Swager, T. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 7486–7488.
13. Zhang, Y.; Yu, J. B.; Gallina, M. E.; Sun, W.; Rong, Y.; Chiu, D. T. *Chem. Commun.* **2013**, 8256–8258.
14. (a) Gao, B. X.; Li, H. X.; Liu, H. M.; Zhang, L. C.; Bai, Q. Q.; Ba, X. W. *Chem. Commun.* **2011**, 3894–3896; (b) Liu, H. M.; Wang, L. Y.; Liu, C. H.; Li, H. X.; Gao, B. X.; Zhang, L. C.; Bo, F. L.; Bai, Q. Q.; Ba, X. W. *J. Mater. Chem.* **2012**, *22*, 6176–6181; (c) Bo, F. L.; Gao, B. X.; Duan, W. F.; Li, H. X.; Liu, H. M.; Bai, Q. Q. *RSC Adv.* **2013**, *3*, 17007–17010.
15. Würthner, F.; Stepanenko, V.; Chen, Z.; Saha-Möller, C. R.; Kocher, N.; Stalke, D. *J. Org. Chem.* **2004**, *69*, 7933–7939.
16. Samudrala, R.; Zhang, X.; Wadkins, R. M.; Mattern, D. L. *Bioorg. Med. Chem.* **2007**, *15*, 186–193.
17. (a) Agrawal, A. K.; Jenekhe, S. A. *Chem. Mater.* **1996**, *8*, 579–589; (b) Yang, C. J.; Jenekhe, S. A. *Macromolecules* **1995**, *28*, 1180–1196; (c) Alam, M. M.; Jenekhe, S. A. *J. Phys. Chem. B* **2002**, *106*, 11172–11177.
18. (a) Bodapati, J. B.; Icil, H. *Dyes Pigm.* **2008**, *79*, 224–235; (b) Langhals, H. *New J. Chem.* **2008**, *32*, 21–23; (c) Heek, T.; Fasting, C.; Rest, C.; Zhang, X.; Würthner, F.; Haag, R. *Chem. Commun.* **2010**, 1884–1886.