DOI: 10.1021/ma102159c

Macromolecules
COMMUNICATION TO THE EDITOR

Synthesis and Characterization of Degradable Water-Soluble Fluorescent Polymers

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Received June 22, 2010
Revised Manuscript Received November 12, 2010

Introduction. Water-soluble conjugated polymers show excellent fluorescence quenching or energy transfer efficiencies in the presence of oppositely charged electron/energy acceptors,[1−4] resulting in the amplification of transduction optical signals of chemical or biological recognition events.[5−15] In recent years, new applications of conjugated polymers in cell imaging, therapy, and biomedical fields have also been successfully explored.[16−18] In contrast to conventional fluorescent organic dyes, conjugated polymers are favorable due to their better photostabilities, higher extinction coefficients, and higher detection sensitivities. However, most of the water-soluble conjugated polymers that have been reported to date are nondegradable.[19] These polymers contain conjugated arylic backbones and are hard to remove from an animal or human body by metabolic paths. So there are potential concerns of toxicity or carcinogenesis when they are used *in vivo*. On the contrary, biodegradable polymers seldom suffer from these problems. There is thus a need to develop degradable water-soluble fluorescent conjugated polymers.

One promising strategy is to develop degradable polymers with fluorescent and water-soluble characteristics. So far, most reported degradable fluorescent polymers[20−24] need to conjugate or encapsulate organic dyes or quantum dots to facilitate their visual detection or monitoring. Unfortunately, most of these polymers exhibit cytotoxicity and poor photostability.[20,25] Previous work reported that[19,26] nonconjugated polymers, where nonconjugated spacers connect the conjugated chromophores, can maintain the fluorescent properties of conjugated polymers except for tuning their energy bands. In this paper, we describe the synthesis and characterization of a novel water-soluble nonconjugated polymer (PMFT) that is obtained by polymerization of a fluorescence generating diol monomer with terephthalic acid. The fluorescent property can be contributed from the fluorescence generating units, and the water solubility can be obtained by introducing a charged group in the side chain of the polymer. Fragile ester bonds, which are easy to break up, are used as nonconjugated spacers. The PMFT is easily degraded under basic conditions at a fast rate. In a physiological environment (pH = 7), PMFT hydrolyzes at a slow rate. The slow degradation pattern with the enhancement of fluorescence intensity of PMFT upon degradation shows a potential *in vivo* application in biological and biomedical fields, especially in triggering drug controlled release.

Experimental Section. *Instruments*. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AV400 instrument. MALDI-TOF and ESI spectra were recorded on Bruker AutoFlexIII and SHIMADZU LCMS 2010 systems, respectively. Elemental analysis was supplied by Flash EA1112. The gel permeation chromatography (GPC) measurements were achieved on a Waters 410 system against polystyrene standards using tetrahydrofuran (THF) as eluent. Florescence spectra were performed on a Hitachi F-4500 fluorometer equipped with a xenon lamp as excitation source at room temperature, and the PMT voltage was 400 V. The fluorescence quantum yields (QYs) were determined with quinine sulfate (0.1 M H_2SO_4, QY = 0.577) as reference.[27] UV−vis spectra were recorded on a Jasco V-500 spectrophotometer. The absorbance at 490 nm for MTT assay was recorded on a microplate reader (BioTek Synergy HT). The UV viewing cabinet used in this study was from ShangHai GuCun Optic Instruments Factory, model ZF-20D, equipped with two 8 W 254 nm and two 8 W 365 nm UV lights. The pH indication was carried on a Mettler Toledo Delta 320 pH indicator, calibrated with standard buffer of pH = 7.00 at room temperature.

Materials. 9,9-Bis(6′-bromohexyl)-2,7-dibromofluorene (**1**) was prepared according to the literature procedure.[2] 4-(Hydroxylmethyl)benzeneboronic acid (97%) and quinine sulfate dehydrate (98%) were purchased from Alfa-Aesar. Terephthaloyl chloride (>99%) was purchased from J&K Chemical. Sodium deuteroxide solution (40 wt % in D_2O, 99+ atom % D) was purchased from Sigma-Aldrich. $PdCl_2$(dppf) (98%) was obtained from Pacific ChemSource, Inc. (China), and 1-methylimidazole (>98%) was purchased from Beijing Ouhe Technology Co., Ltd. (China). All the reagents and solvents were used as received except where noted otherwise. THF was dried over refluxing with sodium and benzophenone as an indicator. Pyridine was dried by refluxing with P_2O_5 for 24 h and distilled in a dry environment. The water used in the experiment was filtered by a Millipore filtration system. The buffer solutions were prepared by 0.1 M Na_2HPO_4 and 0.1 M NaOH and indicated by the pH indicator. Renal cell carcinoma (A498) was purchased from cell center of Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China). Dulbecco's modified Eagles medium (DMEM, high glucose, without phenol red) and fetal bovine serum were purchased from Hyclone/Thermofisher (Beijing, China).

Synthesis of 2,7-Di(4′-phenylmethanol)-9,9-bis(6′-bromohexanyl)fluorene (***3***). The mixture of compound **1** (1.25 g, 1.87 mmol) and compound **2** (760 mg, 5 mmol) in THF (10 mL) and 2.0 M aqueous K_2CO_3 solution (8 mL) was charged with argon for 30 min. Then 40 mg of $PdCl_2$(dppf) (dppf = 1,1′-bis(diphenylphosphine)ferrocene) was added under an argon atmosphere, and the mixture was then stirred at 70 °C for 24 h. After cooling down to room temperature, THF was removed in vacuum from the mixture. The crude product was extracted with 50 mL of $CHCl_3$ for three times. The combined organic phase was washed with brine and dried over anhydrous $MgSO_4$, and the solvent was removed under vacuum. The residue was purified by silica gel column chromatography using ethyl acetate/petroleum ether 1/4 (v/v) mixture as eluent to obtain white solids (684 mg, 52%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.78 (d, J = 7.8 Hz, 2H), 7.69 (d, J = 7.5 Hz, 4H), 7.60 (d, J = 7.8 Hz, 2H), 7.56 (s, 2H), 7.49 (d, J = 7.5 Hz, 4H), 4.77 (s, 4H), 3.26 (t, J = 6.6 Hz, 4H),

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 Published on Web 11/23/2010

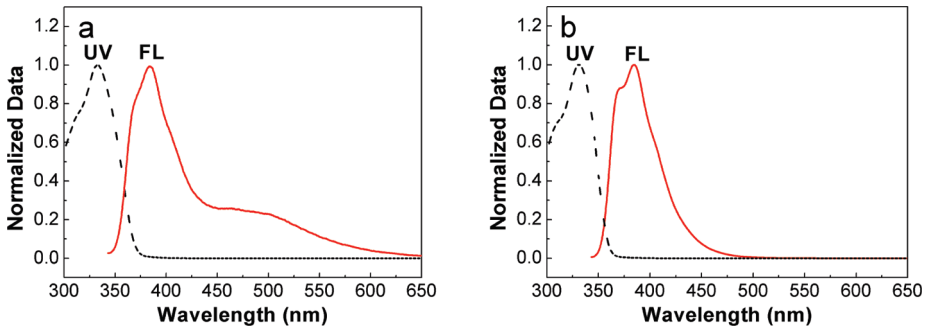
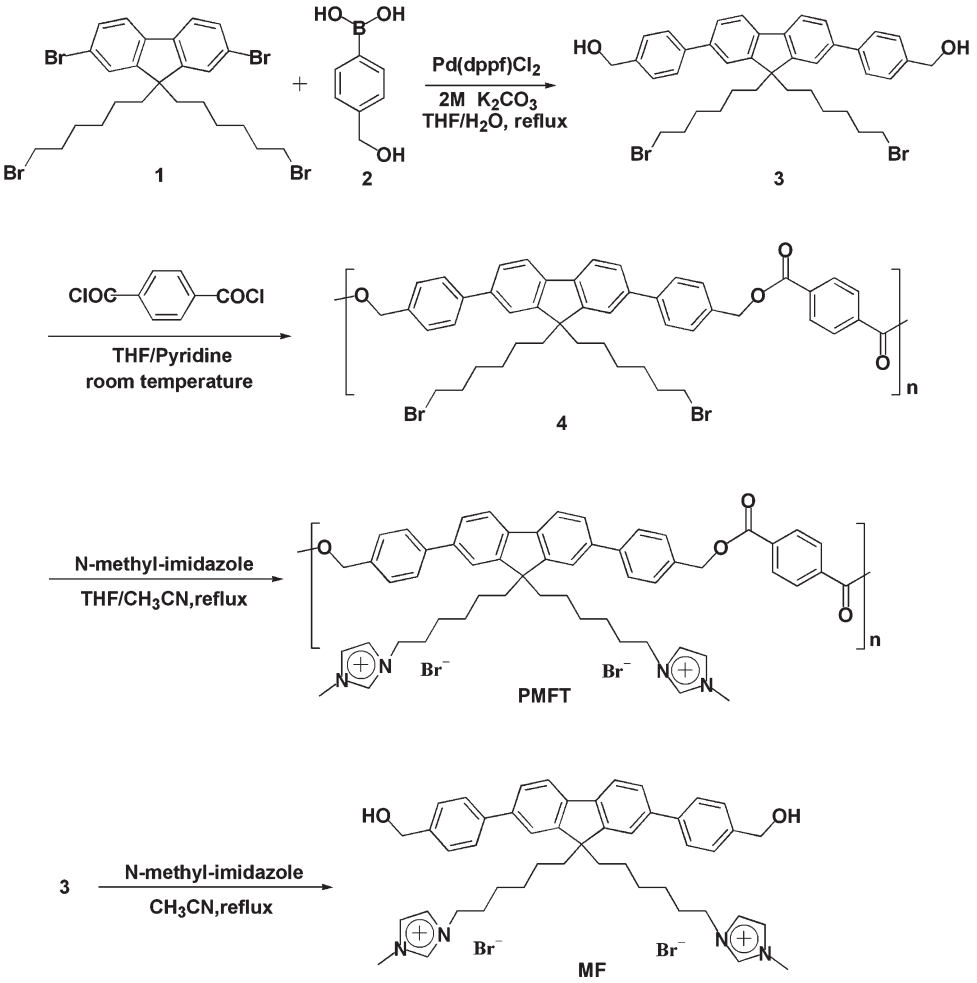


Figure 1. Normalized absorption (black dashed line) and fluorescence spectra (red line) of PMFT (a) and MF (b) in water. [PMFT] = 1.0×10^{-5} M in RUs; excitation wavelength is 333 nm.

Scheme 1. Synthetic Routes for Water-Soluble PMFT and MF



2.07−2.03 (m, 4H), 1.74 (s, 2H), 1.64 (dd, J = 13.9 Hz, 6.9, 4H), 1.20 (dd, J = 14.1 Hz, 7.1 Hz, 4H), 1.10−1.07 (m, 2H), 0.73 (s, 2H). ^{13}C NMR (100 MHz, CD_3OD) δ: 151.33, 140.40, 140.23, 140.12, 139.87, 136.00, 127.28, 126.76, 125.90, 120.92, 120.10, 74.50, 63.65, 33.11, 32.39, 28.61, 27.34, 23.72, 23.43, 19.71. MS (MALDI-TOF): m/z = 704.5 (M + 2H). Anal. Calcd for $C_{39}H_{44}Br_2O_2$: C, 66.48; H, 6.29. Found: C, 66.27; H, 6.45.

Synthesis of Poly(2,7-di(4′-phenylmethanol)-9,9-bis(6′-bromohexanyl))fluorenylene Terephthalate (PFT) (**4**). To a solution of compound **3** (420 mg, 0.6 mmol) in 2 mL of dry THF was added terephthaloyl chloride (150 mg, 0.74 mmol), and the mixture was stirred vigorously for 15 min. Then 0.4 mL of dry pyridine was injected into the reaction solution. The resulting solution was stirred at room temperature for 24 h. The solution was poured into 20 mL of H_2O, and the precipitate was collected by a centrifuge. The crude product was dissolved in 5 mL of $CHCl_3$ and precipitated in 25 mL of methanol and then collected by a centrifuge. The precipitation procedure was repeated three times to yield pale white powder (200 mg, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.24 (br, 2H), 8.17 (br, 2H), 7.79 (br, 2H), 7.70 (br, 4H), 7.65−7.47 (m, 8H), 5.45 (br, 4H), 3.25 (br, 4H), 2.04 (br, 4H), 1.63 (br, 6H), 1.19 (br, 4H), 1.08 (br, 4H), 0.71 (br, 4H). M_n = 4700, M_w = 11 800, and PDI = 2.50 based on GPC analysis.

Synthesis of Poly(2,7-di(4′-phenylmethanol)-9,9-bis(6′-(3″-methylimidazolium)hexanyl)fluorenylene Terephthalate) (PMFT). A solution of PFT (150 mg, 0.032 mmol) and 1-methylimidazole (2 mL, 25 mmol) in 5 mL of CH_3CN and 2 mL of THF was stirred at 65 °C for 24 h. Upon cooling down to room temperature, the solvent and 1-methylimidazole were removed under vacuum. The residue was dissolved in 3 mL of methanol and precipitated in 20 mL of ethyl acetate to get a crude product. The precipitation procedure was repeated twice to obtain a yellow solid (36 mg, 20%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.96 (br, 2H), 8.17 (br, 4H), 8.08 (br, 2H), 7.92 (br, 2H), 7.79 (br, 6H), 7.70 (br, 2H), 7.60 (br, 8H), 5.47 (br, 4H), 3.97 (br, 2H), 3.75 (br, 6H), 2.11 (br, 4H), 1.53 (br, 6H), 1.05 (br, 4H), 0.96 (br, 4H), 0.56 (br, 4H).

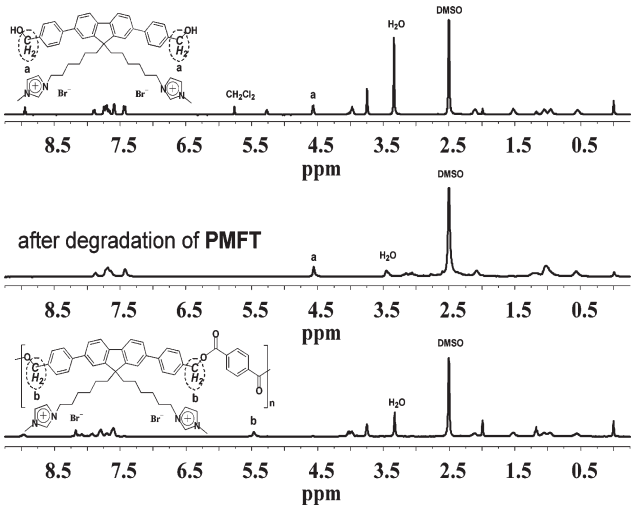


Figure 2. ^{1}H NMR spectra of monomer MF and PMFT before and after treatment with NaOD in DMSO-d_6.

Synthesis of 2,7-Di(4′-phenylmethanol)-9,9-bis(6′-(3″-methylimidazolium)hexanyl)fluorene (MF). A solution of compound **3** (50 mg, 0.07 mmol) and 1-methylimidazole (1 mL, 12.5 mmol) in 10 mL of CH_3CN was stirred at 70 °C for 48 h. Upon cooling down to room temperature, the solvent and 1-methylimidazole were removed under vacuum. The residue was washed by ethyl acetate for three times to afford a yellow solid (42 mg, 80%). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.94 (s, 2H), 7.90 (d, J = 7.7, 2H), 7.79−7.64 (m, 8H), 7.59 (s, 4H), 7.43 (d, J = 7.5, 4H), 5.27 (t, J = 5.2, 2H), 4.57 (d, J = 4.9, 4H), 3.97 (t, J = 6.4, 4H), 3.75 (br, 6H), 2.11 (br, 4H), 1.52 (br, 4H), 1.05 (d, J = 5.4, 4H), 0.95 (br, 4H), 0.55 (br, 4H). MS (ESI): m/z = 354.1 (M−2Br).

PMFT Degradation Experiments. (1) NMR experiment: The PMFT (2 mg) was filled into one NMR tube followed by injection of 550 μL of DMSO-d_6 solvent, and the ^{1}H NMR spectrum was recorded. Then 50 μL of 40 wt % NaOD solution was added to the PMFT solution. After the solution was incubated at room temperature for 24 h, the ^{1}H NMR spectra were recorded again. (2) Fluorescence experiment: To determine change in emission intensity of PMFT upon adding NaOH solution, the emission intensity at 384 nm was recorded as a function of recording time. Six buffer solutions with various pH (10.00, 11.00, 11.30, 11.70, 12.00, 13.00) were made by 100 mM Na_2HPO_4 and 100 mM NaOH and indicated by pH indicator. To 1980 μL of the buffer solution in the cuvette was quickly added 20 μL of PMFT solution in water (1 mM) under vigorous stirring, and the spectra measurement began simultaneously at room temperature up to 1800 s with the fixed excitation wavelength of 333 nm. The procedure was repeated three times. A degradation experiment at physiological pH (pH = 7) was conducted. 10 μL of the PMFT stock solution was injected into 990 μL of double distilled water in the cuvette (pH = 7) followed by incubation at 37 °C. The cuvette cap was sealed tightly to prevent water evaporation. The fluorescence spectrum was recorded every 24 h, until the emission intensity reached a plateau.

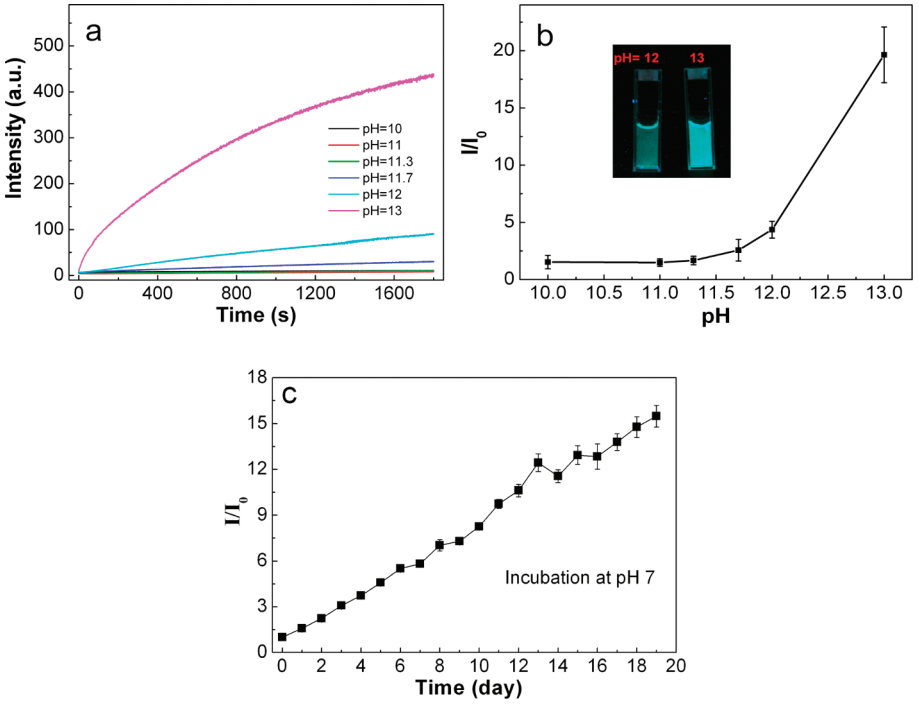


Figure 3. (a) Emission intensity of PMFT versus incubation time in buffer solutions with various pH values. (b) Relative emission intensity of PMFT at 384 nm as a function of pH values. Inset: fluorescence photos of PMFT under UV light after treatment using buffer solutions with various pH values for 10 min. (c) Relative emission intensity of PMFT at 384 nm as a function of incubation time in phosphate buffer at 37 °C (100 mM, pH = 7). [PMFT] = 1.0×10^{-5} M in RUs. The excitation wavelength is 333 nm.

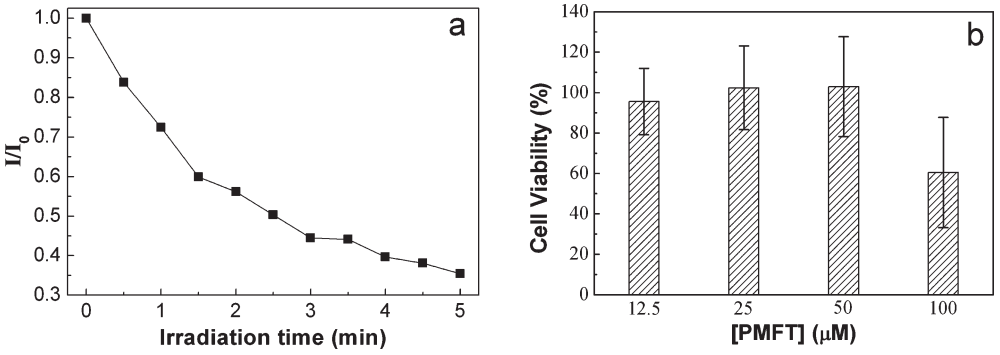


Figure 4. (a) Photostability of PMFT under the irradiation of 365 nm UV light. (b) Cell viability as a function of PMFT concentration. In this experiment, A498 cells were treated with PMFT for 24 h.

Photostability. 20 μL of PMFT stock solution was added into 1980 μL of double distilled water in a quartz cuvette. The cuvette was mixed sufficiently, and the fluorescence spectrum was recorded. Then the cuvette was placed in a UV viewing cabinet. The distance between the UV light and the cuvette was 11.5 cm and the 365 nm light as irradiation source. The total irradiation time was 5 min, and every 30 s the cuvette was taken out to record a fluorescence spectrum.

Cell Viability Assay by MTT. A498 cells were seeded in a flat bottom 96-well tissue culture plate with Dulbecco's modified Eagles medium (DMEM, high glucose, without phenol red) with 10% fetal bovine serum at 37 °C. The plate was incubated under 5% CO_2 humidified atmosphere overnight. Cells were incubated with PMFT with different concentrations for 24 h at 37 °C. Then the cell cultures were discarded, and MTT solution (0.5 mg/mL in DMEM, 100 μL/well) was added into the wells followed by incubation at 37 °C for another 4 h. The supernatant was removed, and 100 μL of DMSO was added into each well and the plate was shaken for 10 min. The absorbance of formazan at 490 nm was recorded on a microplate reader for calculating the cytotoxicity.[28]

Results and Discussion. The synthetic routes for water-soluble polymer PMFT and monomer MF are outlined in Scheme 1. Monomer **3** is prepared by condensation of compound **1** with compound **2** via a Suzuki coupling reaction in the presence of catalyst $PdCl_2$(dppf) in 52% yield. Coupling reaction of compound **3** with terephthaloyl chloride in dry THF and dry pyridine at room temperature for 24 h gives precursor polymer **4** in 40% yield. The polymer **4** has weight-average molecular weight (M_w) of 11 800 with the polydispersity index (PDI = M_w/M_n) of 2.50. The polymer **4** is then reacted with 1-methylimidazole in CH_3CN at 70 °C to afford water-soluble PMFT in 20% yield. Reaction of compound **3** with 1-methylimidazole in CH_3CN affords water-soluble monomer MF in 80% yield. The PMFT is readily dissolved in water containing 5% DMSO (v/v) at room temperature (e.g., 1 mg/mL). The PMFT stock solution will be diluted at least 10 times in cell experiments; thus, the 0.5% DMSO in the solution has no effect on cells *in vitro*.

PMFT is nonconjugated and is composed of fluorescent emitting monomer; thus, the optical properties of both PMFT and monomer MF are measured (Figure 1). The absorption maximum of PMFT is exhibited at 333 nm, which is similar to that of monomer MF. The similar extinction coefficients are also obtained for PMFT (2.51×10^4 M^{-1} cm^{-1}) and monomer MF (3.81×10^4 M^{-1} cm^{-1}). These results show that the maximum absorption of PMFT is corresponding to the $\pi-\pi^*$ transition of the conjugated chromophore. As shown in Figure 1, PMFT and MF exhibit the same emission maxima at 384 nm in aqueous solution. It is noted that a new broad peak from 450 to 550 nm appears for PMFT. These phenomena are in agreement with intermolecular aggregation of conjugated polymers. PMFT is composed of hydrophobic backbones and hydrophilic side chains. The amphiphilic characteristic shows its tendency to form intermolecular aggregations in aqueous solutions. The higher fluorescence quantum efficiency is obtained for monomer MF (76%) rather than polymer PMFT (2%), which results from the fact that the intermolecular $\pi-\pi$ stacking leads to fluorescence self-quenching of the polymer.[29,30]

Structurally, the backbone of PMFT is connected by ester bonds between fluorescent monomer diols and terephthalates. The ester bonds are easily hydrolyzed upon addition of strong bases such as NaOH. Upon treatment with NaOH, the monomer MF and terephthalic acid would release from the polymer. To verify the degradable property of PMFT, the ^1H NMR spectra of PMFT before and after treatment with NaOH were measured. That of monomer MF was also recorded for comparison. As illustrated in Figure 2, the proton chemical shift of $-CH_2-$ in the benzyl alcohol group locates at 4.56 ppm in monomer MF. For the polymer PMFT, the chemical shift of corresponding proton shifts to 5.47 ppm. Upon addition of NaOD to PMFT solution in the NMR tube, the proton chemical shift of $-CH_2-$ in benzyl group neighboring to ester bond in the backbone returns to 4.56 ppm that corresponds to benzyl alcohol group in MF, indicating the ester bond break in the polymer main chain.

Another evidence on PMFT degradation was given by fluorescence spectra. Since fluorescence quantum efficiency of monomer MF (76%) is much higher than that of polymer PMFT (2%), the release of MF from the polymer main chain by PMFT degradation will lead to the enhancement of emission intensity of PMFT solution. To demonstrate this intensity change, time scan mode emission spectra of PMFT were recorded in buffer solutions with various pH values (from 10 to 13). As depicted in Figure 3, the fluorescence enhancement was observed simultaneously upon addition of PMFT solution to NaOH solution. The enhancement rate is in positive correlation with pH value of the buffer; that is, high-pH environment causes PMFT degradation in a rather quick manner. And as time elapses, the degradation process went completely. To offer the direct vision of PMFT degradation, fluorescence photos of PMFT were taken under UV light after treatment using buffer solutions with various pH values (12 and 13) for 10 min. As seen in the inset of Figure 3b, the PMFT solution exhibits bright blue-green emission color in high-pH solution after degradation. These fluorescence results confirm the NMR experiments. To test the ability of PMFT for *in vivo* use, its degradation behavior at physiological condition (pH = 7, temperature = 37 °C) was investigated. As shown in Figure 3c, the PMFT degraded in phosphate buffer (100 mM, pH = 7), but at a rather slow rate

than when under strong base conditions. The hydrolysis proceeded in the time range from 0 to 19 days. The slow degradation pattern with enhancement of fluorescence intensity of PMFT shows a potential *in vivo* application in biological field, especially in drug controlled release.

The photostability of PMFT was studied under UV irradiation with 365 nm as light source. As depicted in Figure 4a, although the fluorescence intensity of PMFT at 384 nm decreased with the irradiation, the fluorescence remained 40% upon continuously irradiating for 4 min. Thus, the fairly good photostability of PMFT makes it possible for fluorescence imaging use. Because of the importance for *in vivo* use, the biocompatibility of PMFT was studied by a typical MTT assay method.[28] As shown in Figure 4b, the PMFT exhibits little cytotoxicity as the concentration increases from 12.5 to 50 μM. Even at high PMFT concentration (100 μM), the cell viability still remains about 60%. The degradation products of PMFT are the monomer MF and terephthalic acid, where terephthalic acid shows little cytotoxicity; thus, the cytotoxicity of MF was also studied. Even at high MF concentration (50 μM), the cell viability still remains above 60%, so we believe that both polymer and degradable products have little cytotocixity in a certain range of concentration. In comparison with the cytotoxicity of quantum dots (QDs)[25] and photobleaching properties of organic dyes, the PMFT has both acceptable cytotoxicity and photostability.

Conclusions. A new water-soluble degradable polyester consisting of fluorescent 2,7-di(4′-phenylmethanol)-9,9-bis-(6′-(3″-methylimidazolium)hexanyl)fluorene chromophore and degradable ester spacer in backbone (PMFT) has been designed and successfully synthesized via facile operations. The fluorescence of the PMFT is from a fluorescent phenylfluorenyl moiety, and its water solubility is obtained by introducing charged groups in the side chain of the polymer. The PMFT is easily degraded in basic conditions, which was confirmed by ^{1}H NMR spectra and fluorescence measurement. Degradation of PMFT was verified by ^{1}H NMR spectroscopy through following the proton chemical shift of the benzyl $-CH_2-$ group adjacent to the ester moiety in PMFT (5.47 ppm) to 4.56 ppm for the methylene proton resonance in the MF degration product. Since fluorescence quantum efficiency of monomer MF (76%) is much higher than that of polymer PMFT (2%), the release of MF from the polymer main chain by PMFT degradation leads to the enhancement of emission intensity of PMFT solution. In physiological environment (pH = 7), PMFT hydrolyzes at a rather slow rate than when under strong basic conditions (10 min). Because of its fluorescent and degradable properties, PMFT may have various *in vivo* applications in biological and biomedical fields such as drug delivery and cell imaging.

Acknowledgment. The authors are grateful for the National Natural Science Foundation of China (Nos. 20725308, 21033010, and 90913014).

References and Notes

(1) Thomas, S. W., III; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339.
(2) Liu, B.; Bazan, G. C. *Chem. Mater.* **2004**, *16*, 4467.
(3) Ho, H. A.; Najari, A.; Leclerc, M. *Acc. Chem. Res.* **2008**, *41*, 168.
(4) Duan, X.; Liu, L.; Feng, F.; Wang, S. *Acc. Chem. Res.* **2010**, *43*, 260.
(5) Ho, H. A.; Dore, K.; Boissinot, M.; Bergeron, M. G.; Tanguay, R. M.; Boudreau, D.; Leclerc, M. *J. Am. Chem. Soc.* **2005**, *127*, 12673.
(6) Nilsson, K. P. R.; Inganas, O. *Nature Mater.* **2003**, *2*, 419.
(7) Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 10954.
(8) Wang, S.; Gaylord, B. S.; Bazan, G. C. *J. Am. Chem. Soc.* **2004**, *126*, 5446.
(9) Wang, D. L.; Gong, X.; Heeger, P. S.; Rininsland, F.; Bazan, G. C.; Heeger, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 49.
(10) Jiang, H.; Taranekar, P.; Reynolds, J. R.; Schanze, K. S. *Angew. Chem., Int. Ed.* **2009**, *48*, 4300.
(11) Xu, H.; Wu, H. P.; Huang, F.; Song, S. P.; Li, W. X.; Cao, Y.; Fan, C. H. *Nucleic Acids Res.* **2005**, *33*, e83.
(12) Bunz, U. H. F. *Chem. Rev.* **2000**, *100*, 1605.
(13) Kim, I. B.; Wilson, J. N.; Bunz, U. H. F. *Chem. Commun.* **2005**, 1273.
(14) Achyuthan, K. E.; Bergstedt, T. S.; Chen, L.; Jones, R. M.; Kumaraswamy, S.; Kushon, S. A.; Ley, K. D.; Lu, L.; McBranch, D.; Mukundan, H.; Rininsland, F.; Shi, X.; Xia, W.; Whitten, D. G. *J. Mater. Chem.* **2005**, *15*, 2648.
(15) Guo, Z. Q.; Zhu, W. H.; Tian, H. *Macromolecules* **2010**, *43*, 739.
(16) Feng, X. L.; Tang, Y. L.; Duan, X. R.; Liu, L. B.; Wang, S. *J. Mater. Chem.* **2010**, *20*, 1312.
(17) Moon, J. H.; McDaniel, W.; MacLean, P.; Hancock, L. E. *Angew. Chem., Int. Ed.* **2007**, *46*, 8223.
(18) McRae, R. L.; Phillips, R. L.; Kim, I. B.; Bunz, U. H. F.; Fahrni, C. J. *J. Am. Chem. Soc.* **2008**, *130*, 7851.
(19) Yang, J.; Zhang, Y.; Gautam, S.; Liu, L.; Dey, J.; Chen, W.; Mason, R. P.; Serrano, C. A.; Schug, K. A.; Tang, L. P. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 11818.
(20) Gao, X. H.; Yang, L. L.; Petros, J. A.; Marshal, F. F.; Simons, J. W.; Nie, S. M. *Curr. Opin. Biotechnol.* **2005**, *16*, 63.
(21) Gaumet, M.; Gurny, R.; Delie, F. *Int. J. Pharm.* **2007**, *342*, 222.
(22) Ogura, Y.; Kimura, H. *Surv. Ophthalmol.* **1995**, *39*, S17.
(23) Ghoroghchian, P. P.; Frail, P. R.; Li, G. Z.; Zupancich, J. A.; Bates, F. S.; Hammer, D. A.; Therien, M. J. *Chem. Mater.* **2007**, *19*, 1309.
(24) Rhyner, M. N.; Smith, A. M.; Gao, X. H.; Mao, H.; Yang, L. L.; Nie, S. M. *Nanomedicine* **2006**, *1*, 209.
(25) Jamieson, T.; Bakhshi, R.; Petrova, D.; Pocock, R.; Imani, M.; Seifalian, A. M. *Biomaterials* **2007**, *28*, 4717.
(26) Zheng, S. Y.; Shi, J. M.; Mateu, R. *Chem. Mater.* **2000**, *12*, 1814.
(27) Lakowicz, J. R. *Principles of Fluoresence Spectoscopy*, 3rd ed.; Springer: New York, 2006.
(28) Denizot, F.; Lang, R. *J. Immunol. Methods* **1986**, *89*, 271.
(29) Arnt, L.; Tew, G. N. *J. Am. Chem. Soc.* **2002**, *124*, 7664.
(30) Chu, Q.; Pang, Y. *J. Polym. Sci., Part A: Polym. Chem.* **2006**, *44*, 2338.