Langmuir
Article
pubs.acs.org/Langmuir

Novel Fluorescent pH Sensors and a Biological Probe Based on Anthracene Derivatives with Aggregation-Induced Emission Characteristics

Hongguang Lu, Bin Xu, Yujie Dong, Feipeng Chen, Yaowen Li, Zaifang Li, Jiating He, Hui Li, and Wenjing Tian*

State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, PR China

Received October 28, 2009. Revised Manuscript Received January 13, 2010

Three functionalized 9,10-distyrylanthracene (DSA) derivatives, namely, 9,10-bis(4-hydroxystyryl)anthracene (**2**), 9,10-bis{4-[2-(diethylamino)ethoxy]styryl}anthracene (**4**), and 9,10-bis{4-[2-(*N*,*N*,*N*-triethylammonium)ethoxy]-styryl}anthracene dibromide (**5**), were synthesized and their fluorescence properties were investigated. The three DSA derivatives possess a typical aggregation-induced emission (AIE) property (i.e., they are nonluminescent in dilute solutions but are efficiently fluorescent as induced by molecular aggregation). Different AIE properties were tuned through molecular structure control. Dye **2** is a phenol-moiety-containing compound, which shows aggregation at pH values smaller than 10, resulting in a high fluorescence intensity. Thus, dye **2** has a pK_a of 9.94. **4** is an amine-containing compound that starts to aggregate at slightly basic conditions, resulting in a pK_a of 6.90. Dye **5** is an ammonium-salt-containing compound. Because it is very soluble in water, this compound has no AIE phenomenon but can interact strongly with protein or DNA to amplify its emission. Therefore, **5** is a fluorescent turn "on" biological probe for protein and DNA detection and it is also selective, which works for native BSA and ct DNA but not their denatured forms. Therefore, we not only developed a few new compounds showing the AIE phenomena but also controlled the AIE through environmental stimulation and demonstrated that the new AIE molecules are suitable for pH and biomacromolecule sensing.

Introduction

Fluorescence (FL) chemo-/biosensors have received a great deal of attention because of their potential applications in chemistry, materials science, biology, and medicine.[1] The FL-based technique offers high sensitivity, low background noise, and wide dynamic ranges.[2] When sensors bind with ions or neutral organic or inorganic molecules, their FL can be enhanced/quenched and/or hypsochromically/bathochromically-shifted, thus enabling the visual observation of the analytes. However, FL dyes tend to aggregate when dispersed in aqueous media or when bound to the analytes in large quantities. The aggregation often quenches FL, which results in drastic reductions in their FL signals. This aggregation-caused quenching (ACQ) has been a thorny problem in the development of efficient FL sensing systems, especially in bioassays of trace numbers of biomolecules.[3] Thus, there is a high demand for the development of simple, stable FL sensors without ACQ.

Recently, materials with aggregation-induced emission (AIE) properties have attracted more and more attention because they offer an efficient path to the solution of this spiny problem of ACQ. Since the first AIE-active material, 1-methyl-1,2,3,4,5-pentaphenylsilole, was reported by Tang's group,[4] many AIE-active dyes have been developed by various research groups, such as siloles,[5] CN-MBE,[6] DPDSB derivatives,[7] DPDBF derivatives,[8] conjugated polymers,[9] and others.[10] The AIE dyes are highly emissive in the solid state and hence are mainly used for the construction of organic light-emitting diodes. In the past few years, the application of AIE molecules to building new chemo-/biosensors has attracted significant scientific interest. Typical AIE molecules are tetraphenylethylene (TPE) derivatives, which can

*Corresponding author. E-mail: wjtian@jlu.edu.cn. Fax: +86-431-85193421.

(1) (a) Haugland, R. P. *Handbook of Fluorescent Probes and Research Chemicals*; Molecular Probes: Leiden, The Netherlands, 2002. (b) Valeur, B. *Molecular Fluorescence: Principles and Applications*; Wiley-VCH: Weinheim, Germany, 2002. (c) Nilsson, K. P. R.; Inganäs, O. *Nat. Mater.* **2003**, *2*, 419. (d) Tian, Y.; Chen, C.-Y.; Yang, C.-C.; Young, A. C.; Jang, S.-H.; Chen, W.-C.; Jen, A. K.-Y. *Chem. Mater.* **2008**, *20*, 1977.

(2) (a) Ambrose, W. P.; Goodwin, P. M.; Jett, J. H.; Orden, A. V.; Werner, J. H.; Keller, R. A. *Chem. Rev.* **1999**, *99*, 2929. (b) Patton, W. F. *Biotechniques* **2000**, *28*, 944.

(3) (a) Berggren, K.; Steinberg, T. H.; Lauber, W. M.; Carroll, J. A.; Lopez, M. F.; Chernokalskaya, E.; Zieske, L.; Diwu, Z.; Haugland, R. P.; Patton, W. F. *Anal. Biochem.* **1999**, *276*, 129. (b) Suzuki, Y.; Yokoyama, K. *J. Am. Chem. Soc.* **2005**, *127*, 17799. (c) Royer, C. A. *Chem. Rev.* **2006**, *106*, 1769.

(4) Luo, J.; Xie, Z.; Lam, J. W. Y.; Cheng, L.; Chen, H.; Qiu, C.; Kwok, H. S.; Zhan, X.; Liu, Y.; Zhu, D.; Tang, B. *Chem. Commun.* **2001**, 1740.

(5) (a) Mi, B.; Dong, Y.; Li, Z.; Lam, J. W. Y.; Häussler, M.; Sung, H. H. Y.; Kwok, H. S.; Dong, Y.; Williams, I. D.; Liu, Y.; Luo, Y.; Shuai, Z.; Zhu, D.; Tang, B. *Chem. Commun.* **2005**, 3583. (b) Yu, G.; Yin, S.; Liu, Y.; Chen, J.; Xu, X.; Sun, X.; Ma, D.; Zhan, X.; Peng, Q.; Shuai, Z.; Tang, B.; Zhu, D.; Fang, W.; Luo, Y. *J. Am. Chem. Soc.* **2005**, *127*, 6335.

(6) (a) An, B.-K.; Kwon, S.-K.; Jung, S.-D.; Park, S. Y. *J. Am. Chem. Soc.* **2002**, *124*, 14410. (b) Lim, S.-J.; An, B.-K.; Jung, S.-D.; Chung, M.-A.; Park, S. Y. *Angew. Chem., Int. Ed.* **2004**, *43*, 6346. (c) An, B.-K.; Gihm, S. H.; Chung, J. W.; Park, C. R.; Kwon, S.-K.; Park, S. Y. *J. Am. Chem. Soc.* **2009**, *131*, 3950.

(7) (a) Xie, Z.; Yang, B.; Cheng, G.; Liu, L.; He, F.; Shen, F.; Ma, Y.; Liu, S. *Chem. Mater.* **2005**, *17*, 1287. (b) Li, Y.; Li, F.; Zhang, H.; Xie, Z.; Xie, W.; Xu, H.; Li, B.; Shen, F.; Ye, L.; Hanif, M.; Ma, D.; Ma, Y. *Chem. Commun.* **2007**, 231.

(8) (a) Tong, H.; Dong, Y.; Häussler, M.; Lam, J. W. Y.; Sung, H. H.-Y.; Williams, I. D.; Sun, J.; Tang, B. *Chem. Commun.* **2006**, 1133. (b) Tong, H.; Dong, Y.; Hong, Y.; Häussler, M.; Lam, J. W. Y.; Sung, H. H.-Y.; Yu, X.; Sun, J.; Williams, I. D.; Kwok, H. S.; Tang, B. *J. Phys. Chem. C* **2007**, *111*, 2287.

(9) (a) Deans, R.; Kim, J.; Machacek, M. R.; Swager, T. M. *J. Am. Chem. Soc.* **2000**, *122*, 8565. (b) Holzer, W.; Penzkofer, A.; Stockmann, R.; Meysel, H.; Liebegott, H.; Hörhold, H. H. *Polymer* **2001**, *42*, 3183. (c) Qin, A.; Jim, C. K. W.; Tang, Y.; Lam, J. W. Y.; Liu, J.; Mahtab, F.; Gao, P.; Tang, B. *J. Phys. Chem. B* **2008**, *112*, 9281.

(10) (a) Kim, S.; Zheng, Q.; He, G. S.; Bharali, D. J.; Pudavar, H. E.; Baev, A.; Prasad, P. N. *Adv. Funct. Mater.* **2006**, *16*, 2317. (b) Kim, S.; Ohulchanskyy, T. Y.; Pudavar, H. E.; Pandey, R. K.; Prasad, P. N. *J. Am. Chem. Soc.* **2007**, *129*, 2669. (c) Tong, H.; Hong, Y.; Dong, Y.; Ren, Y.; Häussler, M.; Lam, J. W. Y.; Wong, K. S.; Tang, B. *J. Phys. Chem. B* **2007**, *111*, 2000. (d) Xu, J.; Liu, X.; Lv, J.; Zhu, M.; Huang, C.; Zhou, W.; Yin, X.; Liu, H.; Li, Y.; Ye, J. *Langmuir* **2008**, *24*, 4231. (e) Qin, A.; Lam, J. W. Y.; Mahtab, F.; Jim, C. K. W.; Tang, L.; Sun, J.; Sung, H. H. Y.; Williams, I. D.; Tang, B. *Appl. Phys. Lett.* **2009**, *94*, 253308.

be used as FL turn-on bioprobes for protein, DNA detection,[11] G-quadruplex formation, and real-time monitoring of DNA folding.[12] Other AIE-active compounds such as silole derivatives have been applied in the FL turn-on detection of heparin in serum,[13] DNA and label-free fluorescence nuclease assay,[14] continuous on-site label-free ATP fluorometric assay,[15] and pH sensing.[16] The AIE dyes used for the FL sensors exhibit the following advantages: (i) the AIE dyes do not suffer from the problem of ACQ and hence extend the effective ranges of the sensors; (ii) most of the sensors based on the AIE feature, in which the FL intensities are turned on from an initially low level, could be more sensitive and faster;[17] and (iii) in contrast to the traditional molecular sensors utilizing different photophysical processes such as photoinduced electron transfer (PET), photoinduced charge transfer (PCT), and excimer/exciplex formation, the AIE-active sensor operates with a new working mechanism that could offer some new possibilities for species detection. The addition of analyte could induce the AIE-active sensors to aggregate or deaggregate, leading to a change in the fluorescence optical signals and thus accomplishing analyte detection. For example, Zhang et al. have established a simple AIE-active probe for in situ, continuous, qualitative adenosine triphosphate (ATP) detection based on the positively charged quaternary ammonium modified silole, which can aggregate on the negatively charged ATP template through charge−charge interaction and emit strong fluorescence in aqueous solution.[15] In addition, they have recently reported a fluorescence turn-on sensing ensemble for cyanide in aqueous solution by making use of the AIE feature of the silole compound.[18] However, until now, studies utilizing AIE dyes as FL sensors have mainly concentrated on TPE derivatives and silole derivatives, so developing new AIE-active sensory materials would be very rewarding work.[19]

Anthracene and its derivatives constitute a very famous class of fluorophores that have been widely used in the development of FL sensors because of their excellent photoluminescence properties and chemical stability.[20] We have recently reported a class of 9,10-distyrylanthracene (DSA) derivatives with AIE properties.[21] The investigation indicates that the restricted intramolecular rotations between the 9,10-anthylene core and the vinylene segment are the cause of the AIE phenomenon. These dye molecules, showing typical AIE characteristics and excellent photoluminescence properties, could be promising candidates as FL sensory materials. Herein, we synthesized three new AIE-active DSA derivatives; dyes **2** and **4** and water-soluble cationic salt **5**; and explored their utility as pH sensors and biological probes on the basis of their AIE characteristics. The FL sensors based on compounds **2** and **4** exhibit well-defined on-and-off behavior in response to the change in pH. Salt **5** is an excellent fluorescent turn-on biological probe for protein and DNA detection and it is also selective, which works for native BSA and ct DNA but not their denatured forms.

Experimental Section

General. All reagents and starting materials are commercially available and were used as received. 9,10-Dibromoanthracene was purchased from Acros and used without further purification. Dimethyl acetamide (DMAc) and tetrahydrofuran (THF) were purified by fractional distillation before use as solvents. As shown in Scheme 1, 1-methoxy-4-vinylbenzene was prepared according to literature procedures.[22] Bovine serum albumin (BSA; > 98%, fraction V) and calf thymus (ct) DNA were purchased as lyophilized crystalline powders from Sigma.

The ^{1}H and ^{13}C NMR spectra were recorded at 298 K on an AVANCZ 500 spectrometer and a Varian Mercury-300 NMR, respectively, with tetramethylsilane (TMS) as the standard. Mass spectra were recorded on an Agilent 1100 LC-MS system. Elemental analysis was performed with a Flash EA 1112 elemental analyzer. UV−vis absorption spectra were recorded on a Lambda-800 spectrophotometer. Fluorescence measurements were carried out with RF-5301PC. Scanning electron microscope (SEM) images were obtained with a field-emission scanning electron microscope (FE-SEM, JEOL JSM-6700F).

UV and FL Spectra. The stock solution of **4** was 1.0×10^{-4} M in acetonitrile. A sample mixture used to measure the UV and FL spectra was prepared by adding 1 mL of a stock solution to 9 mL of acetonitrile or water under vigorous stirring at room temperature. The mixture was stirred for half an hour prior to recording its spectrum. Similarly, BSA was dissolved in a pH 7.0 phosphate buffer solution (0.1 and 1.0 mg/mL), and DNA was dissolved in deionized water (0.1 and 1.0 mg/mL). The stock solution of **5** was 2.5×10^{-4} M in water. FL titration was carried out by adding aliquots of BSA or DNA solution to 0.1 mL of a stock solution of **5**, followed by adding an aqueous phosphate buffer (10 mM, pH 7.0) to acquire a 10.0 mL solution. The mixture was stirred for half an hour prior to recording its spectrum. The relative fluorescence quantum yield (Φ_F) was determined by the standard method using quinine sulfate in 0.1 N H_2SO_4 solution as a reference.

DNA Cleavage Reaction with the Hydroxyl Radical. A total volume of 1 mL of a reaction mixture containing ct DNA (10.0 μg/mL), $FeSO_4$ (14 μM), and H_2O_2 (3.6 mM) was placed in a tube. The mixture was incubated at 37 °C for 30 min and then was added to a buffer solution of **5** (2.5×10^{-6} M in 10 mM phosphate buffer solution, pH 7.0) for FL spectral measurements.

Synthesis of 9,10-Bis(4-methoxystyryl)anthracene (1). A round-bottomed flask (25 mL) was oven dried and cooled under a N_2 atmosphere. 1-Methoxy-4-vinylbenzene (0.32 g, 2.4 mmol), 9,10-dibromoanthracene (0.34 g, 1 mmol), K_3PO_4 (0.64 g, 3 mmol), and $Pd(OAc)_2$ were dissolved in 10 mL of dry DMAc. The reaction mixture was heated to 110 °C in an oil bath and stirred for 24 h at this temperature. After being cooled to room temperature, the reaction mixture was poured into water and extracted with CH_2Cl_2 (3 × 40 mL). The combined organic extracts were washed with brine, dried (Mg_2SO_4), and concentrated to dryness under vacuum. The crude product was purified by silica gel chromatography (4:1 petroleum ether/CH_2Cl_2) to give a yellow solid (0.29 g, 65% yield). ^{1}H NMR ($CDCl_3$, 500 MHz): δ (TMS) 8.39−8.41 (m, 4H), 7.79 (d, J = 16.5 Hz, 2H), 7.63 (d, J = 8.5 Hz, 4H), 7.45−7.47 (m, 4H), 7.00 (d, J = 8.5 Hz, 4H), 6.88 (d, J = 16.5 Hz, 2H), 3.89 (s, 6H). ^{13}C NMR ($CDCl_3$, 75

(11) (a) Tong, H.; Hong, Y.; Dong, Y.; Häussler, M.; Lam, J. W. Y.; Li, Z.; Guo, Z.; Guo, Z.; Tang, B. *Chem. Commun.* **2006**, 3705. (b) Tong, H.; Hong, Y.; Dong, Y.; Häussler, M.; Li, Z.; Lam, J. W. Y.; Dong, Y.; Sung, H. H.-Y.; Williams, I. D.; Tang, B. *J. Phys. Chem. B* **2007**, *111*, 11817.

(12) Hong, Y.; Häussler, M.; Lam, J. W. Y.; Li, Z.; Sin, K. K.; Dong, Y.; Tong, H.; Liu, J.; Qin, A.; Renneberg, R.; Tang, B. *Chem. Eur. J.* **2008**, *14*, 6428.

(13) Wang, M.; Zhang, D.; Zhang, G.; Zhu, D. *Chem. Commun.* **2008**, 4469.

(14) Wang, M.; Zhang, D.; Zhang, G.; Tang, Y.; Wang, S.; Zhu, D. *Anal. Chem.* **2008**, *80*, 6443.

(15) Zhao, M.; Wang, M.; Liu, H.; Liu, D.; Zhang, G.; Zhang, D.; Zhu, D. *Langmuir* **2009**, *25*, 676.

(16) Li, Z.; Dong, Y.; Lam, J. W. Y.; Sun, J.; Qin, A.; Häussler, M.; Dong, Y.; Sung, H. H. Y.; Williams, I. D.; Kwok, H. S.; Tang, B. *Adv. Funct. Mater.* **2009**, *19*, 1.

(17) (a) Mortellaro, M. A.; Nocera, D. G. *J. Am. Chem. Soc.* **1996**, *118*, 7414. (b) McQuade, D. T.; Hegedus, A. H.; Swager, T. M. *J. Am. Chem. Soc.* **2000**, *122*, 12389. (c) Ma, G.; Cheng, Q. *Langmuir* **2006**, *22*, 6743.

(18) Peng, L.; Wang, M.; Zhang, G.; Zhang, D.; Zhu, D. *Org. Lett.* **2009**, *11*, 1943.

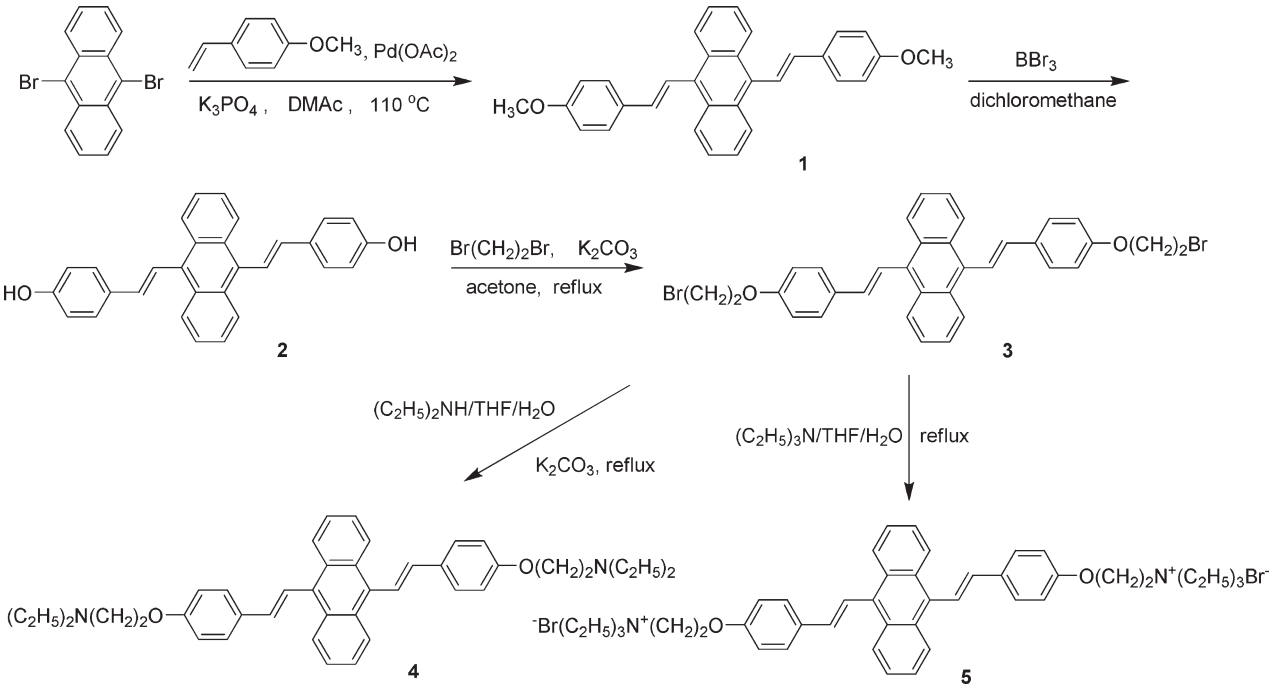
(19) (a) Ning, Z.; Chen, Z.; Zhang, Q.; Yan, Y.; Qian, S.; Cao, Y.; Tian, H. *Adv. Funct. Mater.* **2007**, *17*, 3799. (b) Yuan, C.; Tao, X.; Wang, L.; Yang, J.; Jiang, M. *J. Phys. Chem. C* **2009**, *113*, 6809.

(20) (a) de Silva, A. P.; NimalGunaratne, H. Q.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515. (b) Martinez-Manez, R.; Sancenon, F. *Chem. Rev.* **2003**, *103*, 4419.

(21) He, J.; Xu, B.; Cheng, F.; Xia, H.; Li, K.; Ye, L.; Tian, W. *J. Phys. Chem. C* **2009**, *113*, 9892.

(22) Xia, H.; He, J.; Peng, P.; Zhou, Y.; Li, Y.; Tian, W. *Tetrahedron Lett.* **2007**, *48*, 5877.

Scheme 1. Synthesis of Functionalized DSA Derivatives 2, 4, and 5 from 1



MHz): δ (TMS) 159.73, 136.90, 132.83, 130.35, 129.73, 127.83, 126.55, 125.10, 123.05, 114.35, 55.45. EI-MS *m*/*e*: 443.4 ($[M + H]^+$, calcd 443.19). Anal. Calcd for $C_{32}H_{26}O_2$: C, 86.85; H, 5.92. Found: C, 86.71; H, 6.01.

Synthesis of 9,10-Bis(4-hydroxystyryl)anthracene (2). Into a 100 mL flask were added 1.33 g of **1** (3.00 mmol) and 30 mL of dichloromethane (DCM). The flask was placed in an acetone−dry ice bath at −78 °C. A solution of 3.02 g of boron tribromide (12.0 mmol) in 20 mL of DCM was added carefully to the mixture with stirring. The resultant mixture was allowed to warm to room temperature and was stirred overnight. The reaction product was hydrolyzed by careful shaking with 20 mL of water. The organic phase was separated and concentrated by a rotary evaporator. The crude product was purified by silica gel chromatography (3:1 petroleum ether/acetone) to give a yellow solid (1.03 g, 83% yield). ^{1}H NMR (DMSO-d_6, 500 MHz): δ (TMS): 9.67 (s, 2H), 8.37−8.39 (m, 4H), 7.86 (d, J = 16.5 Hz, 2H), 7.63 (d, J = 8.5 Hz, 4H), 7.53−7.55 (m, 4H), 6.85 (d, J = 8.5 Hz, 4H), 6.80 (d, J = 16.5 Hz, 2H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ (TMS): 157.61, 137.03, 132.36, 128.98, 128.09, 128.06, 126.22, 125.31, 121.11, 115.55. EI-MS *m*/*e*: 415.2 ($[M + H]^+$, calcd 415.16). Anal. Calcd for $C_{30}H_{22}O_2$: C, 86.93; H, 5.35. Found: C, 86.79; H, 5.46.

Synthesis of 9,10-Bis[4-(2-bromoethoxy)styryl]anthracene (3). Into a 100 mL round-bottomed flask were added 3.31 g of K_2CO_3 (24.0 mmol), 1.00 g of **2** (2.40 mmol), and 9.02 g of 1,2-dibromoethane (48.0 mol) in 40 mL of dry acetone. The mixture was heated to reflux and stirred for 24 h. After filtration and concentration, the crude product was purified by silica gel chromatography (2:1 petroleum ether/CH_2Cl_2) to give a yellow solid (0.79 g, 52% yield). ^{1}H NMR ($CDCl_3$,500 MHz): δ (TMS) 8.38−8.40 (m, 4H), 7.79 (d, J = 16.5 Hz, 2H), 7.62 (d, J = 8.5 Hz, 4H), 7.46−7.49 (m, 4H), 7.00 (d, J = 8.5 Hz, 4H), 6.86 (d, J = 16.5 Hz, 2H), 4.38 (t, 4H), 3.70 (t, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ (TMS): 158.08, 136.69, 132.73, 130.98, 129.63, 127.91, 126.50, 125.17, 123.41, 115.15, 68.05, 29.02. EI-MS *m*/*e*: 629.1 ($[M + H]^+$, calcd 629.04); Anal. Calcd for $C_{34}H_{28}Br_2O_2$: C, 64.99; H, 4.49. Found: C, 64.81; H, 4.47.

Synthesis of 9,10-Bis{4-[2-(diethylamino)ethoxy]styryl}-anthracene (4). Into a 100 mL round-bottomed flask were added 1.05 g of K_2CO_3 (7.50 mmol), 3 mL of diethylamine (30.0 mmol), and 0.94 g of **3** (1.50 mmol) in 31 mL of THF/H_2O (30:1 v/v). After being refluxed for 24 h, the mixture was cooled to room temperature and 3 mL of 37% hydrochloric acid and 50 mL of water were added. The mixture was extracted with 100 mL of $CHCl_3$ three times. The organic layers were combined and dried over Mg_2SO_4 overnight. After filtration and solvent evaporation, the crude product was purified by silica gel chromatography (1:1 petroleum ether/CH_2Cl_2) to give a yellow solid (0.58 g, 63% yield). ^{1}H NMR ($CDCl_3$,500 MHz): δ (TMS) 8.39−8.41 (m, 4H), 7.76 (d, J = 16.5 Hz, 2H), 7.60 (d, J = 8.5 Hz, 4H), 7.45−7.47 (m, 4H), 7.00 (d, J = 8.5 Hz, 4H), 6.85 (d, J = 16.5 Hz, 2H), 4.14 (t, 4H), 2.94 (t, 4H), 2.67 (m, 8H), 1.11 (t, 12H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (TMS) 158.86, 136.85, 132.77, 130.24, 129.64, 127.77, 126.52, 125.08, 122.90, 114.89, 66.67, 51.76, 47.85, 11.79. EI-MS *m*/*e*: 613.4 ($[M + H]^+$, calcd 613.37). Anal. Calcd for $C_{42}H_{48}N_2O_2$: C, 82.31; H, 7.89; N, 4.57. Found: C, 82.29; H, 7.92; N, 4.49.

Synthesis of 9,10-Bis{4-[2-(*N*,*N*,*N*-triethylammonium)-ethoxy]styryl}anthracene Dibromide (5). A 100 mL flask with a magnetic spin bar was charged with 0.11 g of **3** (0.17 mmol) dissolved in 50 mL of THF. To this solution was added 5 mL of triethylamine (35.9 mmol). The mixture was heated to reflux and stirred for 3 days. During this period, 10 mL of water was added at several intervals. THF and extra triethylamine were evaporated. The water solution was washed with chloroform three times. After solvent evaporation, the residue was washed with chloroform and acetone and then dried overnight in vacuo at 50 °C. The product was isolated in 48% yield (0.07 g). ^{1}H NMR (DMSO-d_6, 500 MHz): δ (TMS) 8.37−8.39 (m, 4H), 8.00 (d, J = 16.5 Hz, 2H), 7.81 (d, J = 8.5 Hz, 4H), 7.56−7.58 (m, 4H), 7.10 (d, J = 8.5 Hz, 4H), 6.89 (d, J = 16.5 Hz, 2H), 4.48−4.50 (t, 4H), 3.72−3.74 (t, 4H), 3.40−3.45(m, 12H), 1.25−1.28(t, 18H). ^{13}C NMR (DMSO-d_6, 75 MHz): δ (TMS) 157.40, 136.57, 132.34, 130.46, 128.97, 128.15, 126.23, 125.50, 122.82, 114.95, 61.18, 55.22, 52.97, 7.36. EI-MS *m*/*e*: 335.3 ($[M - 2Br]^{2+}$, calcd 335.23). Anal. Calcd for $C_{46}H_{58}Br_2N_2O_2$: C, 66.50; H, 7.04; N, 3.37. Found: C, 66.28; H, 7.27; N, 3.21.

Results and Discussion

Synthesis and Characterization. The DSA derivatives were prepared by the synthesis route shown in Scheme 1. DSA derivative **1** was obtained in all-trans conformations by the Heck

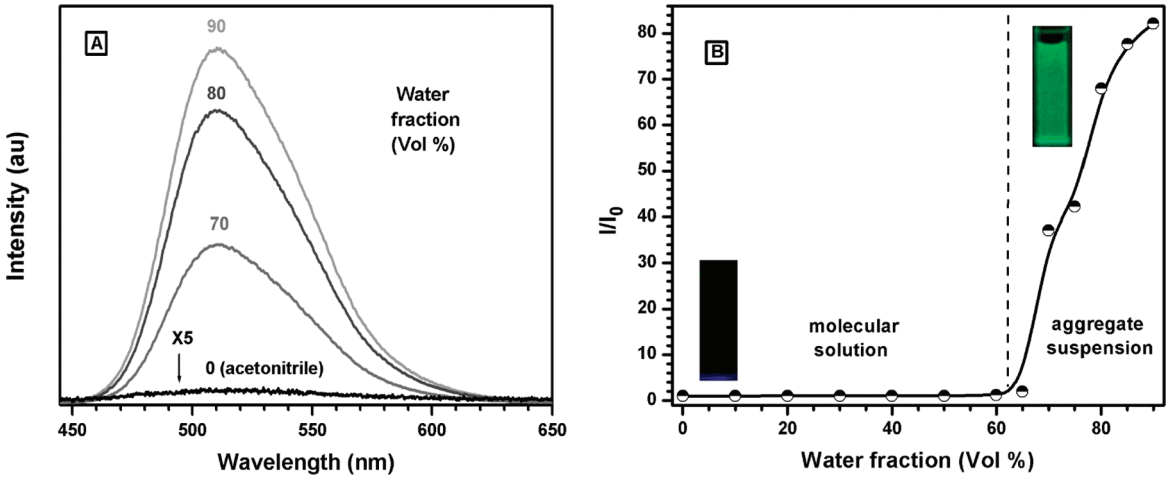


Figure 1. (A) FL spectra of **4** in acetonitrile/water mixtures and (B) dependence of the I/I_0 ratios of **4** on the solvent composition of the acetonitrile/water mixture. The concentration of **4** is 10 μM, and the excitation wavelength is 425 nm.

coupling reaction. The demethylation of **1** by BBr_3 followed by treatment with water gave dihydroxylated DSA derivative **2**. The reactions of **2** with 1,2-dibromoethane in the presence of K_2CO_3 yielded DSA derivative **3**. DSA derivative **4** was synthesized by the reaction of **3** with diethylamine. The quaternization of **3** by NEt_3 gave salt **5**. The structures of the product molecules were confirmed by ^{1}H NMR, ^{13}C NMR, EI-MS, and elemental analysis. Dyes **2** and **4** are soluble in common organic solvents such as acetonitrile (AN), acetone, chloroform, and THF but insoluble in water. Salts **5** are soluble in water as well as methanol, dimethyl formamide (DMF), and dimethyl sulfoxide (DMSO).

AIE Effect. **4** is almost nonluminescent in acetonitrile (AN) with an extremely low fluorescence quantum yield (Φ_F) of 0.25%, and a suspension of **4** in AN/water with a high water fraction larger than 65% is highly emissive (Figure 1A), suggesting strong aggregations of **4** in water-rich solvents. As indicated in Figure S1 (Supporting Information), the absorption of **4** obviously decreases along with the FL emission increase. The formation of nanoscopic aggregates of **4** is suggested by the leveling-off tail in the visible region of the absorption spectrum because of the Mie effect of the nanoparticles.[23] The nanoscopic aggregations of **4** were virtually confirmed by SEM imaging (Figure S2). Nanoparticles with average sizes of ~100−150 nm are clearly observed. Thus, **4** is AIE-active.

The light-scattering effect of nanoaggregates in aqueous mixtures with different water contents makes the Φ_F measurements inaccurate.[10e] We thus used the changes in the FL intensity of **4** in the AN/water mixtures with different water contents to reveal the AIE characteristics. The I/I_0 ratio in the solvent mixtures remains almost unchanged until up to ~65% water is added (Figure 1B). The ratio starts to increase when the dyes begin to cluster together in the water/AN mixtures with >65% water. When the water fraction is increased to 90%, the fluorescence intensity is ~82-fold higher than in pure AN solution (I_0).

Salt **5** is completely soluble in water. The addition of AN, THF, and methanol to the solution of the sample in water fails to make the dye molecules aggregate, possibly because of its amphiphilic nature, which is associated with its binary tetraalkylammonium moieties.[11a] The emission in the mixtures is as weak as that in the pure water solution. However, the increasing viscosity and decreasing temperature of the solution of **5** can increase its FL intensity (Figure 2). At room temperature, the FL intensity of a dilute solution of **5** in a viscous glycerol/water mixture (9:1 v/v) is much higher than that in pure water. As the solution temperature is decreased from 25 to −78 °C, the FL intensity of **5** is increased. These phenomena occur because high viscosity and low temperature can hamper intramolecular rotation, leading to the closure of the nonradiative decay channel and thus enhanced FL emission. The results indicate that the restriction of intramolecular rotation plays a very important role in inducing the dye to emit, which coincides with our previous study on the AIE of the DSA derivatives.[21]

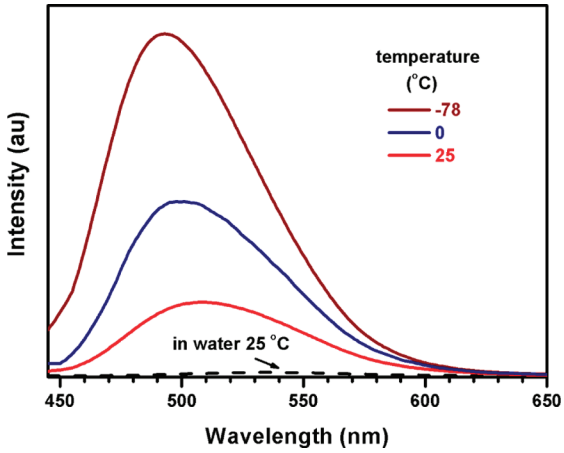


Figure 2. Emission spectra of **5** (2.5 μM) in a glycerol/water mixture with 90% glycerol at −78, 0, and 25 °C. Data for **5** in water (2.5 μM) at 25 °C is shown for comparison.

pH Sensing. Recently, stimuli-sensitive functional materials have received much attention.[24] In particular, fluorescent pH sensors have extensively been pursued in analytical chemistry, bioanalytical chemistry, cellular biology, and medicine.[25] DSA derivatives show a distinct phenomenon of AIE: the nonemissive molecules can be induced to emit efficiently by aggregate formation, which provides an opportunity to design a novel pH sensor by making use of the AIE feature.

(23) Auweter, H.; Haberkorn, H.; Heckmann, W.; Horn, D.; Lüddecke, E.; Rieger, J.; Weiss, H. *Angew. Chem., Int. Ed.* **1999**, *38*, 2188.

(24) (a) Yoshida, R.; Uchida, K.; Kaneko, Y.; Sakai, K.; Kikuchi, A.; Sakurai, Y.; Okano, T. *Nature* (*London*) **1995**, *374*, 240. (b) Roy, I.; Gupta, M. N. *Chem. Biol.* **2003**, *10*, 1161. (c) Yu, X.; Wang, Z.; Jiang, Y.; Shi, F.; Zhang, X. *Adv. Mater.* **2005**, *17*, 1289.

(25) (a) Lee, S.-H.; Kumar, J.; Tripathy, S. K. *Langmuir* **2000**, *16*, 10482. (b) Lee, J. W.; Lee, J.-S.; Chang, Y.-T. *Angew. Chem., Int. Ed.* **2006**, *45*, 6485. (c) Uchiyama, S.; Makino, Y. *Chem. Commun.* **2009**, 2646.

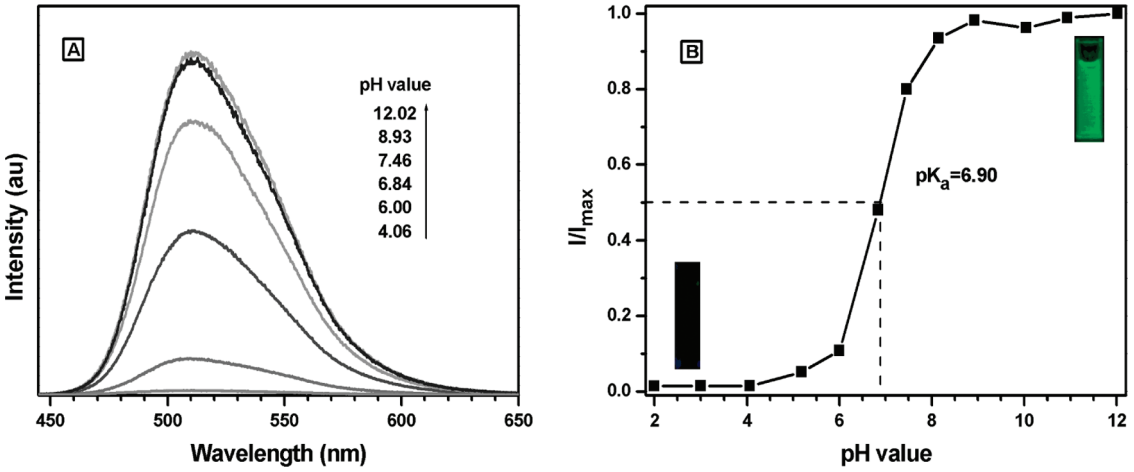


Figure 3. (A) FL spectra of **4** (20 μM) at different pH values in an acetonitrile/water mixture (3:7 v/v). (B) pH dependence of **4** (I/I_{max}) at 296 K in acetonitrile/water mixtures (3:7 v/v) at different pH values.

As discussed above, dye **4** is nonluminescent in acidic solution with pH lower than 4. At pH higher than 4, the FL intensity starts to increase quickly. At a pH of 8.5, the solution emits strongly in the green spectral window at 510 nm and the FL intensity is 70-fold that at pH 3.0 (Figure 3). Furthermore, the difference in the fluorescence intensities of **4** at high and low pH can be easily distinguished by the naked eye under UV light (365 nm) illumination (inset of Figure 3B). Furthermore, the FL intensity of **4** exhibits a distinct increase when the pH increases from 2 to 12, and its emission could be quenched when the pH is reduced from 12 to 2. The reversibility experiments demonstrate that the AIE of compound **4** is reversible when the pH is increased from 2 to 12 and again returns to 2 (Figure S3 in Supporting Information). These phenomena occur because the structure of **4** can be changed from an isolated species to aggregated clusters in response to the variation in pH. At low pH, **4** is converted into an ammonium salt form and thus dissolves in water. The solution is nonemissive. A similar phenomenon was observed for **5** and has been discussed. When the pH is higher than 5, the ammonium salt form of **4** started to convert back to its free amine form, which is insoluble and aggregates in water. Thus, the fluorescence coming from the aggregation state is turned on. The AIE nature makes **4** pH-sensitive.

Different from **4**, compound **2** bearing two phenol substituents behaves in an exactly opposite manner. As shown in Figure 4, the FL emission of **2** is turned on at low pH values and turned off at high pH values, which is attributed to deionization and ionization under acidic and basic conditions, respectively. The phenol substituents may be converted into the quinone structure under basic conditions, but compound **2**, within 2 weeks, cannot converted to the quinone form under basic conditions, as suggested by the fact that the absorption spectra of a basic solution of **2** undergo no distinct change within 2 weeks. Figures 3B and 4 illustrate the pH response of sensors **2** and **4** as a function of I/I_{max} versus pH, where I is the measured FL emission at that pH and I_{max} is the maximum output of the sensor. The pK_a can be estimated where I/I_{max} is 0.5. The pK_a values for **2** and **4** are 9.94 and 6.90, respectively, showing the structural influence on the pH response. Neutral compound **3** also showed strong aggregation in water-rich solvent; however, because **3** has no pH-sensitive moiety, its aggregation state has no significant differences at various pH values, which can be reflected in its quantum yields (Table 1).

Biological Probing. The study of the detection of biomacromolecules by FL probes has received a great deal of attention

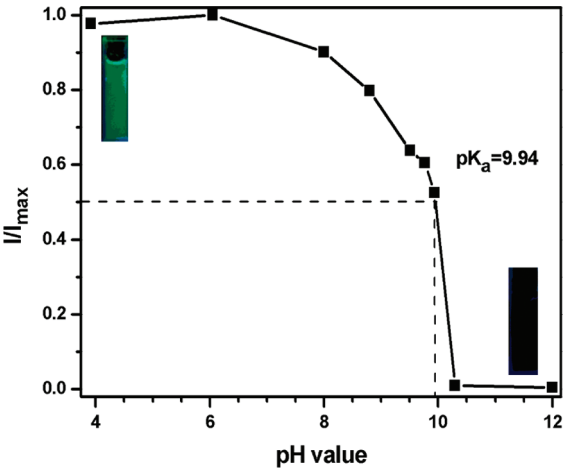


Figure 4. pH dependence of **2** (I/I_{max}) at 296 K in acetonitrile/water mixtures (3:7 v/v) of differing pH.

Table 1. Fluorescence Quantum Yield of DSA Derivatives 2, 3, 4, and 5 under Acidic, Neutral, and Basic Conditions

	Φ_F (%)[a]		
DSA	pH 2	pH 7	pH 12
2	6.87	5.82	0.02
3	12.74	11.76	10.33
4	0.14	9.12	20.40
5	0.19	0.20	0.19

[a] Fluorescence quantum yield (Φ_F) of DSA derivatives **2**, **3**, **4**, and **5** (20 μM) at different pH values in an acetonitrile/water mixture (3:7 v/v).

because of its potential application in biological science and technology.[26] However, the aggregation of dyes causes FL quenching, which limits the effective ranges of the probes. This makes it particularly difficult to assay low-abundance biomacromolecules. The bioprobe using an AIE dye does not suffer from ACQ.

Figure 5A shows the FL spectral changes in **5** before and after the addition of BSA. Water-soluble salt **5** is nonluminescent in a pH 7.0 phosphate buffer; however, the FL intensity of the

(26) (a) Peczuh, M. W.; Hamilton, A. D. *Chem. Rev.* **2000**, *100*, 2479. (b) Medintz, I. L.; Uyeda, H. T.; Goldman, E. R.; Mattoussi, H. *Nat. Mater.* **2005**, *4*, 435. (c) Herland, A.; Inganäs, O. *Macromol. Rapid Commun.* **2007**, *28*, 1703.

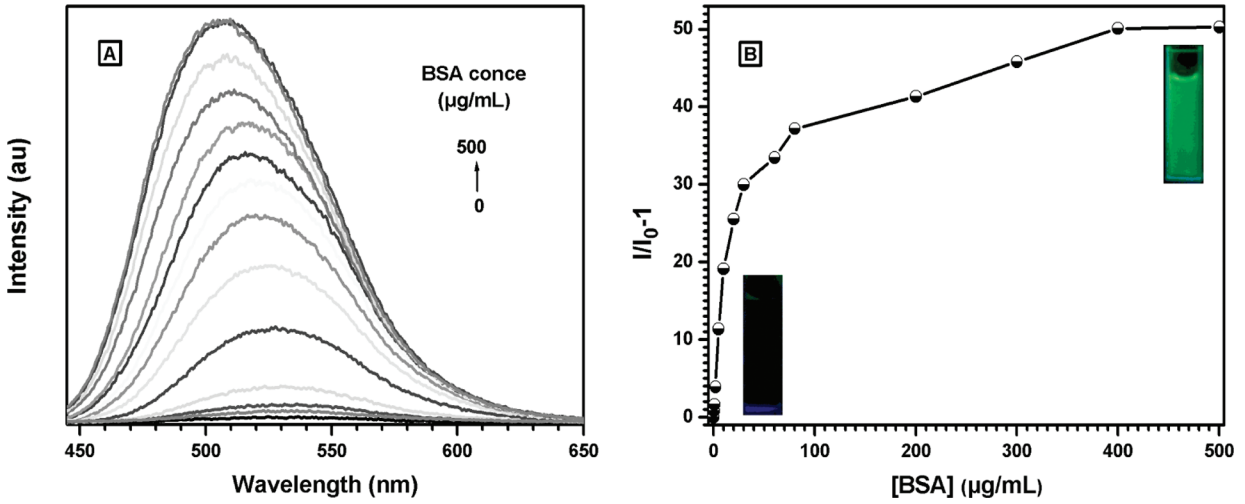


Figure 5. (A) Change in the FL spectrum of **5** (2.5 μM) with the addition of BSA to an aqueous phosphate buffer (pH 7.0). (B) Plot of FL intensity vs BSA concentration.

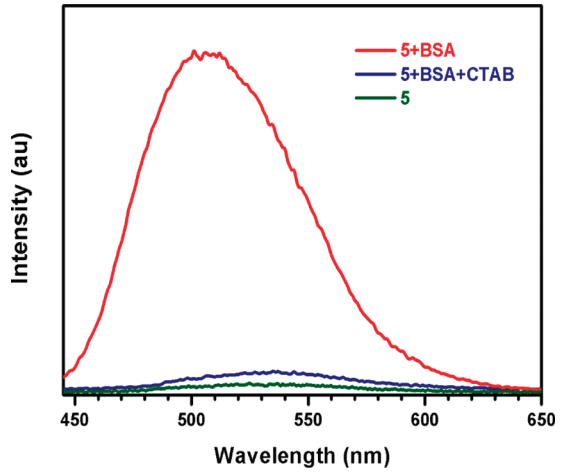


Figure 6. Effect of BSA (100 μg/mL) and/or CTAB (0.6 mg/mL) on the FL spectrum of a buffer solution of **5** (2.5 μM).

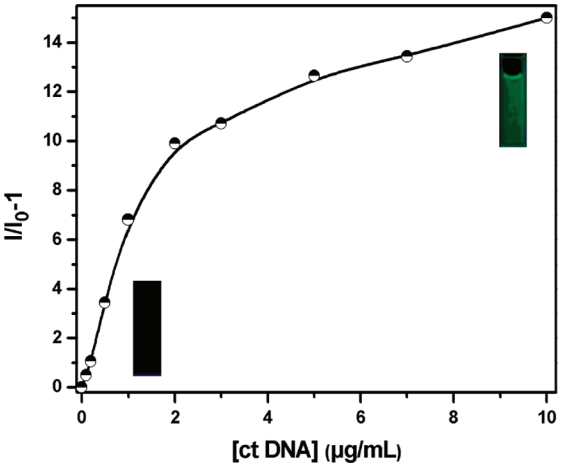


Figure 7. Plot of FL intensity of **5** (2.5 μM) in an aqueous phosphate buffer (pH 7.0) vs ct DNA concentration.

buffer solution of **5** increased gradually when BSA was introduced. For instance, the FL intensity of **5** (2.5 μM) at 510 nm is enhanced by 50-fold after the concentration of BSA reached 500 μg/mL (Figure 5B). The detection limit can reach 500 ng/mL for BSA. Meanwhile, as indicated in Figure 6, the FL of the ensemble of **5** (2.5 μM) and BSA (100 μg/mL) is rather strong; however, it is dramatically weakened after cetyltrimethylammonium bromide (CTAB) is added to the BSA solution of **5**. The probe is thus folding-structure-sensitive because the surfactant molecules of CTAB can unfold the folding structure of BSA chains and destroy the native hydrophobic regions of the protein.[27]

In addition to protein probing, salt **5** is also a sensitive FL bioprobe for DNA detection. The emission of **5** (2.5 μM) is turned on when ct DNA is added to its phosphate buffer solution (pH 7.0, Figure S5 in Supporting Information). The FL intensity of **5** was enhanced 15-fold after the concentration of ct DNA reached 10 μg/mL (Figure 7). Actually, the FL difference in the solution of **5** before and after the addition of ct DNA can be distinguished by the naked eye as displayed in the inset of Figure 7. Moreover, the detection limit can reach 100 ng/mL for ct DNA.

It is well known that Fenton's reagent can generate HO· from Fe^{2+} and H_2O_2 and HO· can cut DNA into different sequence fragments and even single bases.[28] The cleavage process of DNA by HO· can be followed with salt **5**. As indicated in Figure 8, the FL of the ensemble of **5** (2.5 μM in 10 mM phosphate buffer solution, pH 7.0) and ct DNA (10 μg/mL) is very strong; however, if ct DNA is pretreated with Fenton's reagent, which generates HO· to cleave DNA, then the FL of ensemble of **5** and ct DNA fragments becomes very weak. In the meantime, when the cleavage process is performed after ct DNA detection by **5** is completed, the FL of the ensemble of **5** and ct DNA is also decreased distinctly. Clearly, the probe is selective: it works for native DNA but not its fragments. More importantly, apart from HO·, nuclease can also cleave DNA into fragments.[28b] Therefore, it is possible to employ **5** to follow the DNA cleavage process by nucleases, leading to the development of a label-free fluorescence nuclease assay.

Previous studies of DSA derivatives indicated that the restricted intramolecular rotations between the 9,10-anthrylene core and the vinylene moiety are the main origin of the AIE properties.[21] How, then, is the AIE effect of **5** associated with the

(27) (a) Valstar, A.; Almgren, M.; Brown, W.; Vasilescu, M. *Langmuir* **2000**, *16*, 922. (b) Gull, N.; Chodankar, S.; Aswal, V. K.; Sen, P.; Khan, R. H.; Kabir-ud-Dina *Colloids Surf., B* **2009**, *69*, 122.

(28) (a) Shigenaga, M. K.; Park, J.-W.; Cundy, K. C.; Gimeno, C. J.; Ames, B. N. *Methods Enzymol.* **1990**, *186*, 521. (b) Tang, Y.; Feng, F.; He, F.; Wang, S.; Li, Y.; Zhu, D. *J. Am. Chem. Soc.* **2006**, *128*, 14972.

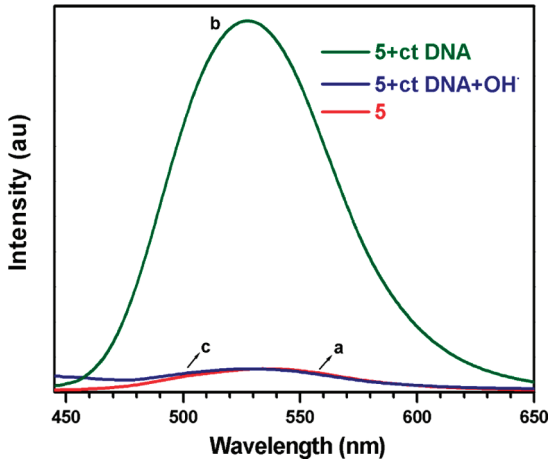


Figure 8. FL spectrum of (a) **5** (2.5 μM in 10 mM phosphate buffer solution, pH 7.0) and those in the presence of (b) ct DNA (10.0 μg/mL) and (c) ct DNA (10.0 μg/mL), which was pretreated with HO· (generated from 14 μM $FeSO_4$ and 3.6 mM H_2O_2) at 37 °C for 30 min.

bioprobing process? In the buffer solutions, the cationic amphiphilic dyes bind to the biomacromolecules (protein and DNA) via supramolecular interactions such as electrostatic interaction and the hydrophobic effect.[11] For the native BSA, it contains hydrophobic binding sites such as hydrophobic pockets. When the dye molecules bind to the hydrophobic regions of BSA chains and enter into the hydrophobic pockets of their folding structures, the intramolecular rotations of the dye molecules are frozen, inducing them to emit as aggregation does. For ct DNA, the formation of a **5**−ct DNA complex is driven by the electrostatic interaction between the ammonium group in **5** and the negative phosphate backbones in ct DNA and the possible hydrophobic interaction between nucleosides and aryl rings in **5**. These intermolecular forces suppress intramolecular rotations of the dye molecules, accordingly activating their AIE processes. Moreover, these conclusions can be proven by the phenomenon in which the emissions of **5** are turned off, when the native structures of biomacromolecules are destroyed (including BSA unfolding by CTAB and DNA cleavage by HO·, see Figures 6 and 8).

Conclusions

In this work, we designed DSA-based new compounds with different AIE aggregation properties through the tuning of chemical structures. Dye **2** is a phenol-moiety-containing compound that shows aggregation at pH values smaller than 10 to result in a high FL intensity with a pK_a of 9.94. **4** is an amine-containing compound that starts to aggregate at slightly basic conditions, resulting in a pK_a of 6.90. Thus, **4** might be suitable for pH sensing in a biological environment. Dye **5** is ammonium-salt-containing compound. Because it is very soluble in water, this compound exhibits no AIE phenomenon, but **5** can interact strongly with protein or DNA to amplify its emission. Therefore, **5** is an excellent fluorescent turn-on biological probe for protein and DNA detection. The detection limits for BSA and ct DNA can reach 500 and 100 ng/mL, respectively. Probe **5** is also selective, which works for native BSA and ct DNA but not their denatured forms. Therefore, we not only developed a few new compounds showing the AIE phenomena but also controlled AIE through environmental stimulation and demonstrated that the new AIE molecules are suitable for pH and biomacromolecular sensing. Moreover, studies on their further applications in biological science and technology are currently in progress.

Acknowledgment. We are grateful to Dr. Yanqing Tian at the Biodesign Institute of Arizona State University for helpful discussions. This work was supported by the 973 Program (2009CB623605), the NSFC (grant no. 50673035), the State Key Development Program for Basic Research of China (grant no. 2009CB623605), the Program for New Century Excellent Talents in Universities of the China Ministry of Education, and the 111 Project (grant no. B06009).

Supporting Information Available: Absorption and emission spectra of **4** in acetonitrile and an acetonitrile/water mixture. SEM image of nanoaggregates of **4** in acetonitrile/water mixtures. FL spectra of **2** at different pH values in an acetonitrile/water mixture. pH dependence of **4** in acetonitrile/water mixtures at differing pH. Change in the FL spectrum of **5** with the addition of ct DNA to an aqueous phosphate buffer. This material is available free of charge via the Internet at http://pubs.acs.org.