Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters 49 (2008) 2656–2660
Tetrahedron Letters

# $Mg^{2+}$-mediated self-assembly of an amphiphilic pyrene derivative with single-stranded DNA

Jinchong Xiao, Junbo Li, Cuihong Li, Changshui Huang,
Yuliang Li *, Shuang Cui, Shu Wang *, Huibiao Liu

*Beijing National Laboratory for Molecular Sciences, Key Laboratory of Organic Solids, Center for Molecular Sciences,*
*Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, PR China*

Received 9 November 2007; revised 3 February 2008; accepted 6 February 2008
Available online 9 February 2008

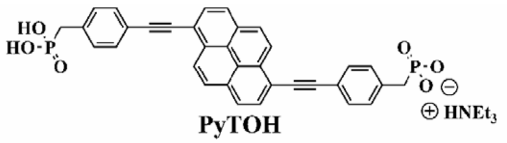
## Abstract

An amphiphilic ethynyl-pyrene derivative modified with phosphonic acid groups (**PyTOH**) was synthesized and characterized. Single-stranded DNA (ssDNA), which carries negative charges, was found to induce the self-assembly of **PyTOH** through electrostatic interactions with $Mg^{2+}$ as co-complexation ion in aqueous solution. UV–vis, fluorescence, and circular dichroism (CD) spectra revealed that left-handed helical architecture of **PyTOH**/$Mg^{2+}$/DNA was formed. Scanning electron microscopy (SEM) and atomic force microscopy (AFM) showed that nanoscale structures could be fabricated by self-assembly.

*Keywords:* Self-assembly; Chiral; ssDNA; Aggregation

Design and synthesis of supramolecular assemblies that mimic the molecular organization in biological systems is of widespread interest, especially in the fields of information storage, light energy conversion, and optical devices.[1] In general, the supramolecular systems can be constructed through weak interactions such as hydrogen-bonding, π–π stacking, electrostatic interaction, van der Waals forces, and solvophobic interactions. DNA-templated fabrication is a significant approach for constructing the supramolecular assemblies, and many advances have been achieved in this field.[2] The molecular recognition features present in DNA have been incorporated into amphiphiles to afford DNA analogs.[3] They have also served as templates for the deposition of conductive materials and silver metallization.[4]

Pyrene and its derivatives exhibit interesting electrochemical and photophysical properties, which have attracted considerable research interests in their aggregation-induced emissions and nanometer-scale molecular architectures.[5] However, they are rarely exploited for fabricating chiral supramolecular architectures with DNA through metal-coordination interactions. Herein, we describe the synthesis of an amphiphilic ethynyl-pyrene (**PyTOH**) containing phosphonic acid groups and the evaluation of its self-assembly behavior with ssDNA in the presence of $Mg^{2+}$ ions in aqueous solutions (Scheme 1). The importance of $Mg^{2+}$ ions has been reported in catalyzing the self-assembly and stabilizing the supramolecular structures.[6]



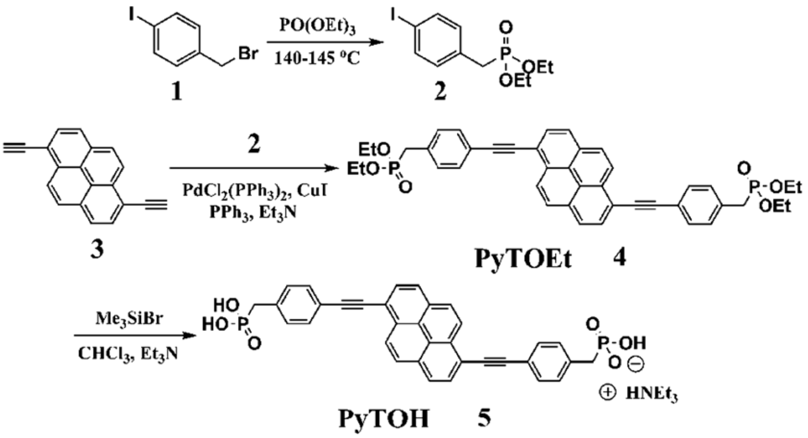
**ssDNA sequence:5'-ATC TTG ACT ATG TGG GTG CTA ACT C-3' 25mer**

Scheme 1. Chemical structure of **PyTOH** and single-stranded DNA.

---

* Corresponding authors. Tel.: +86 10 62588934; fax: +86 10 82616576 (Y.L.).
*E-mail addresses:* steven_zhou1981@hotmail.com, ylli@iccas.ac.cn (Y. Li).

0040-4039/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2008.02.024



Scheme 2. Synthetic routine of **PyTOH**.

The amphiphilic **PyTOH** consists of two phosphonic acid groups, which are linked at both ends of the aromatic ethynyl-pyrene core. The synthesis of **PyTOH** is shown in Scheme 2.[7] Phosphonate (**2**) was afforded from 4-iodobenzyl bromide (**1**) by a Michaelis–Arbuzov reaction, followed by treatment with compounds (**3**) under basic condition to yield the corresponding precursor **PyTOEt** (**4**). The Sonogashira reaction was used to build triple bonds. Cleavage of the ethyl protecting groups afforded the target **PyTOH** (**5**). **PyTOH** was characterized by NMR spectroscopies and ESI mass spectrometry. These spectra indicated that **PyTOH** existed as mono-ammonium salt with triethylamine.

**PyTOH** in water exhibits the absorption maxima at 410 nm with a shoulder peak at 455 nm (for the ethynyl-pyrene core) and the emission maxima at 430 nm upon excitation with 400 nm in dilute solution. The fluorescence quantum yield ($\Phi$) of **PyTOH** in water was measured to be 0.05 using rhodamine 6G as standard. The addition of $Mg^{2+}$ to the solution of **PyTOH** in water resulted in an obvious decrease in the absorbance of the peak at 410 nm and maintained peak position of absorption maximum. The emission intensity was also observed to decrease upon the addition of $Mg^{2+}$ (Fig. 1). The stoichiometry of **PyTOH**/$Mg^{2+}$ complex was determined by the method of continuous variations (Job's plot). The result obtained indicates the formation of a 1:2 **PyTOH**/$Mg^{2+}$ complex.

To investigate the possible expression of chirality within the aggregates and thus gain more information on the structure of the assemblies, absorption, fluorescence, and circular dichroism (CD) experiments were carried out to study the self-assembly behavior of **PyTOH**/$Mg^{2+}$/DNA in aqueous solution. The CD spectrum of ssDNA itself in aqueous solution shows a positive Cotton effect (from 303 nm to 260 nm) followed by a negative Cotton effect at short wavelengths (from 260 nm to 230 nm) (Fig. 2C). The concentration of DNA/$Mg^{2+}$ ([DNA] = $1.0 \times 10^{-4}$ M, 1:2) was kept constant, and the change of the CD spectra of the complex **PyTOH**/$Mg^{2+}$/DNA was followed as a function of increasing concentration of **PyTOH** in 0.1 M Tris/HCl buffer solution (Fig. 2A). At the concentration of **PyTOH** above $3.0 \times 10^{-5}$ M, the CD spectra of the complex showed obvious negative cotton signal from 460 nm to 380 nm, which corresponded to the $\pi$–$\pi^*$ absorption of **PyTOH**. This result indicates that the chirality of **PyTOH** is induced by the DNA upon complexation leading to a left-handed helical stacking of the **PyTOH**.[8] In the control experiments, solutions of **PyTOH**, **PyTOH**/$Mg^{2+}$, and **PyTOH**/DNA were CD-inactive above 350 nm wavelength, which indicated that $Mg^{2+}$ played an important

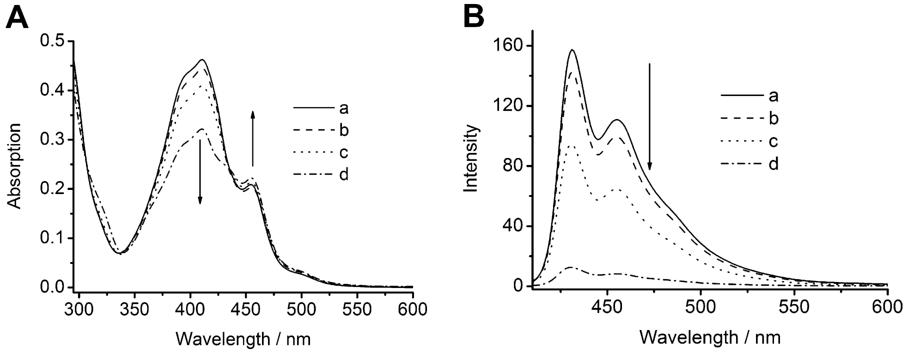


Fig. 1. (A) UV–vis absorption, and (B) fluorescence (excitation at 400 nm) titration of $Mg^{2+}$ to **PyTOH** in 0.1 Tris/HCl buffer solution. [**PyTOH**] = $1.0 \times 10^{-5}$ M, [$Mg^{2+}$] = (a) 0; (b) $5.0 \times 10^{-6}$ M; (c) $1.0 \times 10^{-5}$ M; (d) $2.0 \times 10^{-5}$ M.

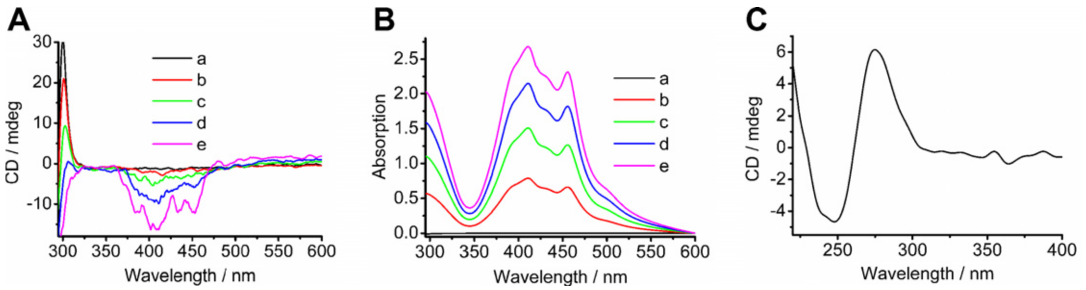


Fig. 2. (A) CD spectra, (B) UV–vis absorption titration of **PyTOH** to DNA/$Mg^{2+}$ in 0.1 Tris/HCl buffer solution. [DNA] = $1.0 \times 10^{-4}$ M, [**PyTOH**] = (a) 0; (b) $3.0 \times 10^{-5}$ M; (c) $6.0 \times 10^{-5}$ M; (d) $8.0 \times 10^{-5}$ M; (e) $1.0 \times 10^{-4}$ M. (C) CD spectrum of ssDNA in buffer solution ($c = 1.0 \times 10^{-5}$ M).

role in catalyzing the self-assembly and stabilizing the supramolecular structures. The self-assembly behavior of **PyTOH**/$Mg^{2+}$/DNA is strongly dependent on the concentration of **PyTOH** used.

We also measured the effect of DNA concentration on the CD signals of **PyTOH**/$Mg^{2+}$/DNA in buffer solution at 15 °C. Although the solution of **PyTOH**/ $Mg^{2+}$ is CD-silent, upon the addition of DNA, the CD spectra with the negative cotton effect at 455, 410 nm was observed (Fig. 3). Noted that the CD signal was not changed with varying DNA concentration. The results indicate that the DNA drives the helical structure of **PyTOH** and contributes to the stability of the complex.

To further take an insight into the self-assembly process among the three building blocks, temperature-dependent optical measurements were carried out for the 1:2:1 complex **PyTOH**/$Mg^{2+}$/DNA in aqueous solution. When the temperature was increased from 5 to 60 °C, the absorbance intensities of the complex **PyTOH**/$Mg^{2+}$/DNA at 410 and 455 nm obviously decreased, but those at 340 nm and 480–680 nm increased (Fig. 4B). While the solution of **PyTOH** in water was cooled down from 60 to 5 °C, the emission intensities were enhanced by probably 25-fold accompanying by a red-shift of 10 nm (Fig. 4C). These results indicated that the rotation of the alkyne-aryl moiety was effectively limited leading to the enhanced emission efficiency when the temperature was decreased.[9] The presence of two well-defined isosbestic points at 367 and 478 nm, respectively, showed the clean conversion of twisted structure to coplanar type. Although it was well known that CD spectra induced by the formation of chiral aggregates were sensitive to temperature,[10] the intensity of CD spectra of the complex **PyTOH**/$Mg^{2+}$/DNA in buffer solution did not change much within the temperature range from 5 to 60 °C. This behavior probably resulted from the strong binding force between **PyTOH** and DNA through $Mg^{2+}$ ions co-coordinating interactions.

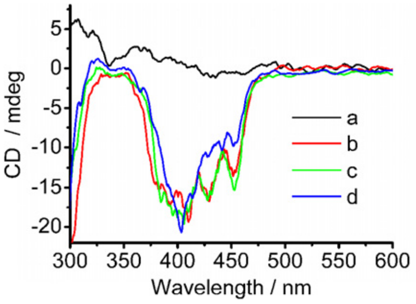


Fig. 3. CD spectra of **PyTOH**/$Mg^{2+}$/DNA as a function of varying DNA concentration in 0.1 Tris/HCl buffer solution. [**PyTOH**] = $1.0 \times 10^{-4}$ M, [DNA] = (a) 0; (b) $4.0 \times 10^{-5}$ M; (c) $6.0 \times 10^{-5}$ M; (d) $1.0 \times 10^{-4}$ M.

The morphologies of the **PyTOH**/$Mg^{2+}$/DNA complex were studied by scanning electron microscopy (SEM) and atomic force microscopy (AFM). Samples were prepared by casting **PyTOH**/$Mg^{2+}$/DNA from water solution onto silicon slices or mica. Figure 5a showed that the complex in water ([**PyTOH**] = $5.0 \times 10^{-5}$ M, [DNA] = $1.0 \times 10^{-4}$ M, [$Mg^{2+}$] = $5.0 \times 10^{-5}$ M) could form the aggregated nanostructures consisting of nanoparticles with diameters in the range of 30–150 nm. The AFM image of the complex also shows the formation of spherical assem-

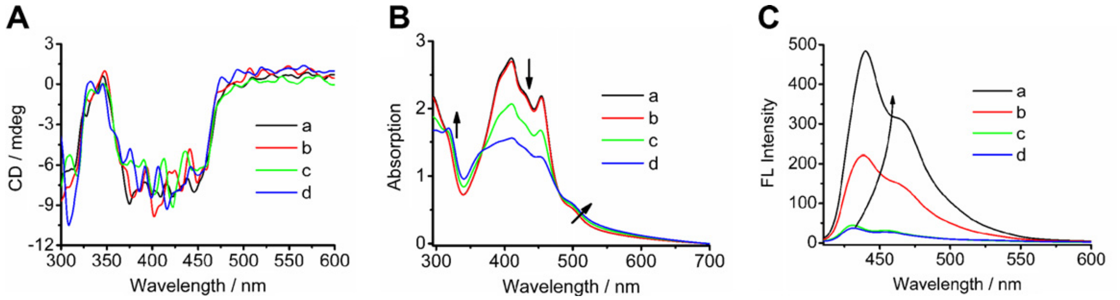


Fig. 4. (A) Temperature dependence of the CD spectra, (B) UV–vis absorption of self-assembly of **PyTOH**/$Mg^{2+}$/DNA ([**PyTOH**] = $1.0 \times 10^{-4}$ M, mol. ratio: 1:2:1), (C) FL spectra ([**PyTOH**] = $1.0 \times 10^{-5}$ M) in 0.1 Tris/HCl buffer solution; (a) 5 °C; (b) 15 °C; (c) 50 °C; (d) 60 °C.

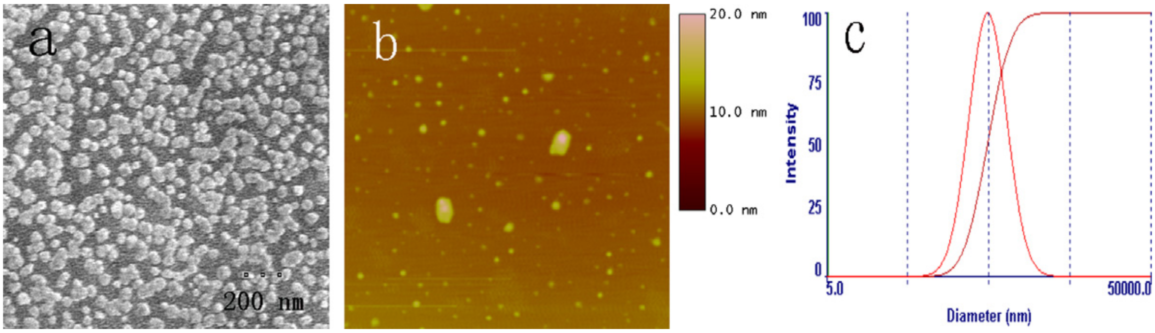


Fig. 5. SEM image of **PyTOH**/Mg$^{2+}$/DNA (mol. ratio, 1:2:1) (a), AFM image (b), and dynamic laser light scattering (c) of **PyTOH**/Mg$^{2+}$/DNA (from CONTIN analysis of the autocorrelation function) at a scattering angle of 90°. Samples were prepared from solution and transferred to fresh silica slice or mica by drop-casting.

blies (Fig. 5b). The size of the aggregates of **PyTOH**/Mg$^{2+}$/DNA in aqueous solution was measured by dynamic light scattering (DLS) (Fig. 5c). The CONTIN analysis of the autocorrelation function shows a broad peak corresponding to an average hydrodynamic radius ($R_h$) of approximately 210 nm at 298 K.

On the basis of the optical and CD properties described above, it can be concluded that the self-assembly of **PyTOH**/Mg$^{2+}$/DNA formed left-handed helical stack structures. The aggregation was initiated by the electrostatic interactions of **PyTOH** and DNA with Mg$^{2+}$ as co-complexation ion in aqueous solution. The mutation rotation in the same direction avoided steric hindrance of **PyTOH** and the aggregation could self-sort into helical nanostructures.

In summary, we have synthesized an amphiphilic pyrene derivative and demonstrated the self-assembly of **PyTOH**/Mg$^{2+}$/DNA in aqueous solution. Induced CD spectra were observed in water indicating the formation of helical supramolecular structures. These observations should facilitate the future design of related self-assembled materials with the biological and electrooptical properties.

## Acknowledgments

This work was supported by the National Natural Science Foundation of China (20531060, 20418001, 20473102) and the Major State Basic Research development Program (2006CB806200, 2005CB623602).

## References and notes

1. (a) Lehn, J. M. *Supramolecular Chemistry, Concepts and Perspectives*; VCH: Weinheim, 1995; (b) Cornelessen, J. J. L. M.; Roman, A. E.; Nolte, R. J. M.; Sommerdijk, N. A. J. M. *Chem. Rev.* **2001**, *101*, 4039; (c) Brunsveld, L.; Folmer, B. J. B.; Meijer, E. W.; Sijbesma, R. P. *Chem. Rev.* **2001**, *101*, 4071; (d) Albrecht, M. *Chem. Rev.* **2001**, *101*, 3457; (e) Hoeben, F. J. M.; Jonkheijm, P.; Meijer, E. W. *Chem. Rev.* **2005**, *105*, 1491; (f) Moreau, L.; Campins, N.; Grinstaff, M. W.; Barthelemy, P. *Tetrahedron Lett.* **2006**, *47*, 7117; (g) Tang, Y. L.; Feng, F. D.; He, F.; Wang, S.; Li, Y. L.; Zhu, D. B. *J. Am. Chem. Soc.* **2006**, *128*, 14972; (h) Janssen, P. G. A.; Vandenbergh, J.; van Dongen, J. L. J.; Meijer, E. W.; Schenning, A. P. H. J. *J. Am. Chem. Soc.* **2007**, *129*, 6078; (i) Gan, H. Y.; Li, Y. L.; Liu, H. B.; Wang, S.; Li, C. H.; Yuan, M. J.; Liu, X. F.; Wang, C. R.; Jiang, L.; Zhu, D. B. *Biomacromolecules* **2007**, *8*, 1713.
2. (a) Storhoff, J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849; (b) Park, S. J.; Lazarides, A. A.; Mirkin, C. A.; Letsinger, R. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 2909; (c) Seeman, N. C. *Nature* **2003**, *421*, 427; (d) Liu, H. P.; He, Y.; Ribbe, A. E.; Mao, C. D. *Biomacromolecules* **2005**, *6*, 2943; (e) Wagner, C.; Wagenknecht, H. A. *Org. Lett.* **2006**, *8*, 4191; (f) Ren, K. F.; Shen, J. C. *Biomaterials* **2006**, *27*, 1152.
3. (a) Onda, M.; Yoshihara, K.; Koyano, H.; Ariga, K.; Kunitake, T. *J. Am. Chem. Soc.* **1996**, *118*, 8524; (b) Berti, D.; Barbaro, P.; Bucci, H.; Baglioni, P. *J. Phys. Chem. B* **1999**, *103*, 4916; (c) Moreau, L.; Barthelemy, P.; El Maataoui, M.; Grinstaff, M. W. *J. Am. Chem. Soc.* **2004**, *126*, 7533; (d) Wang, W.; Li, A. D. Q. *Bioconjugate Chem.* **2007**, *18*, 1036.
4. (a) Braun, E.; Eichen, Y.; Sivan, U.; Ben-Yoseph, G. *Nature* **1998**, *391*, 775; (b) Monson, C. F.; Woolley, A. T. *Nano Lett.* **2003**, *3*, 359; (c) Becerril, H. A.; Stoltenberg, R. M.; Monson, C. F.; Woolley, A. T. *J. Mater. Chem.* **2004**, *14*, 611; (d) Berti, L.; Alessandrini, A.; Facci, P. *J. Am. Chem. Soc.* **2005**, *127*, 11216.
5. (a) Winnik, F. M. *Chem. Rev.* **1993**, *93*, 587; (b) Arimori, S.; Bell, M. L.; Oh, C. S.; James, T. D. *Org. Lett.* **2002**, *4*, 4249; (c) Martin, R. B.; Qu, L. W.; Lin, Y.; Harruff, B. A.; Bunker, C. E.; Gord, J. R.; Allard, L. F.; Sun, Y. P. *J. Phys. Chem. B* **2004**, *108*, 11447; (d) Martinez, R.; Espinosa, A.; Tarraga, A.; Molina, P. *Org. Lett.* **2005**, *7*, 5869; (e) Kamikawa, Y.; Kato, T. *Org. Lett.* **2006**, *8*, 2463; (f) Yang, Z. M.; Xu, B. *J. Mater. Chem.* **2007**, *17*, 2385; (g) Xiao, J. C.; Li, Y. J.; Song, Y. B.; Jiang, L.; Li, Y. L.; Wang, S.; Liu, H. B.; Xu, W.; Zhu, D. B. *Tetrahedron Lett.* **2007**, *48*, 7599; (h) Zhang, S.; Lü, F.; Gao, L.; Ding, L.; Fang, Y. *Langmuir* **2007**, *23*, 1584; (i) Xiao, J. C.; Xu, J. L.; Cui, S.; Liu, H. B.; Wang, S.; Li, Y. L. *Org. Lett.* **2008**, *10*, 645.
6. (a) Dai, T. Y.; Marotta, S. P.; Sheardy, R. D. *Biochemistry* **1995**, *34*, 3655; (b) Yu, H. T.; Hurley, L. H.; Kerwin, S. M. *J. Am. Chem. Soc.* **1996**, *118*, 7040; (c) Rudyak, S. G.; Brenowitz, M.; Shrader, T. E. *Biochemistry* **2001**, *40*, 9317; (d) Bates, A. D.; Callen, B. P.; Cooper, J. M.; Cosstick, R.; Geary, C.; Glidle, A.; Jaeger, L.; Pearson, J. L.; Proupín-Pérez, M.; Xu, C. G.; Cumming, D. R. S. *Nano Lett.* **2006**, *6*, 445.
7. (a) Peeters, E.; van Hal, P. A.; Knol, J.; Brabec, C. J.; Sariciftci, N. S.; Hummelen, J. C.; Janssen, R. A. J. *J. Phys. Chem. B* **2000**, *104*, 10174; (b) Loewe, R. S.; Ambroise, A.; Muthukumaran, K.; Padmaja, K.; Lysenko, A. B.; Mathur, G.; Li, Q.; Bocian, D. F.; Misra, V.; Lindsey, J. S. *J. Org. Chem.* **2004**, *69*, 1453; (c) Viau, L.; Maury, O.; Bozec, H. L. *Tetrahedron Lett.* **2004**, *45*, 125; (d) Leroy-Lhez, S.; Fages, F. *Eur. J. Org. Chem.* **2005**, *2005*, 2684; (e) Compounds **2**, **4**, and **5** were synthesized as follows: Compound **2**: A mixture of triethyl phosphate (1.35 g, 8.11 mmol) and 1-(bromomethyl)-4-iodobenzene (**1**, 2.0 g, 6.76 mmol) was stirred for 4 h at 140–145 °C. The reaction mixture was cooled down to 75 °C, and the excess triethyl phosphate was removed by distillation under reduced pressure to give a white oil **2**

(2.34 g, 97%). $^1$H NMR (300 MHz, $CDCl_3$, 298 K, TMS): $\delta = 7.63$ (d, $J = 7.8$ Hz, 2H), 7.04 (dd, $^1J = 7.8$ Hz, $^2J = 2.1$ Hz, 2H), 4.07–3.97 (m, 4H), 3.07 (d, $J = 21$ Hz, 1H), 1.28–1.23 (t, $J = 7.2$ Hz, 6H). MS (EI): 354 [$M^+$]. Compound **PyTOEt** (**4**): A mixture of 1,6-diethynylpyrene (**3**, 80 mg, 0.32 mmol), compound **2** (248 mg, 0.70 mmol), CuI (3 mg, 0.016 mmol), $PPh_3$ (4 mg, 0.016 mmol), and $Pd(PPh_3)_2Cl_2$ (12 mg, 0.016 mmol) in anhydrous $Et_3N$ (50 mL) was stirred for 12 h at 90 °C under nitrogen. After the mixture was cooled down to room temperature, the mixture was filtrated and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel) with dichloromethane/ethyl acetate as eluent to give **4** (105 mg, 47%). Mp: 163–165 °C; $^1$H NMR (400 MHz, $CDCl_3$, 298 K, TMS): $\delta = 8.67$ (d, $J = 8.8$ Hz, 2H), 8.23–8.15 (m, 6H), 7.67 (d, $J = 8.0$ Hz, 2H), 7.38 (d, $J = 8.0$ Hz, 2H), 4.10–4.03 (m, 8H), 3.22 (d, $J = 22.0$ Hz, 4H), 1.28 (t, $J = 7.2$ Hz, 12H). $^{13}$C NMR (150 MHz, $CDCl_3$, 298 K, TMS): 132.4, 132.3, 132.1, 131.9, 131.3, 130.1, 130.0, 128.3, 126.4, 125.3, 124.3, 122.1, 118.5, 95.4, 88.8, 62.4, 34.5, 33.5, 16.5. $^{31}$P NMR (160 MHz, $CDCl_3$, 298 K, TMS): 25.9. MALDI-TOF MS: Calcd. for $C_{42}H_{40}O_6P_2$: $[M]^+ = 702.2$, $[M+Na]^+ = 725.2$. Found: 702.6 [$M^+$], 725.6 $[M+Na]^+$. HR-MS calcd for $C_{42}H_{40}O_6P_2$ 702.2300; found, 702.2305. Compound **PyTOH** (**5**): **PyTOEt** (**4**, 50 mg, 0.071 mmol) and triethylamine (800 μL, 5.74 mmol) were dissolved in 50 mL of chloroform, and then bromotrimethylsilane (546 μL, 2.13 mmol) was added to the solution. The mixture was stirred overnight under nitrogen at 65 °C. The reaction mixture was cooled down to room temperature, washed with water and extracted with chloroform (20 mL × 3). The organic layer was separated, dried with anhydrous $Na_2SO_4$ and concentrated under reduced pressure. The residue was suspended in hexane and sonicated for 5 min, and then the supernatant was centrifuged. This process was repeated three times. The solution was poured into methanol with sonication, and then was poured into diethyl ether and the precipitate was collected. This process was also repeated three times. The precipitate was collected and dried in vacuum to afford a yellow solid **5** (32 mg, 65%). >200 °C Dec; $^1$H NMR (300 MHz, DMSO-$d_6$, 398 K, TMS): $\delta = 8.44$ (d, $J = 8.7$ Hz, 2H), 8.14–8.06 (m, 6 H), 7.58 (d, $J = 7.5$ Hz, 2H), 7.36 (d, $J = 7.5$ Hz, 2H), 6.47 (br, 3H), 2.97 (d, $J = 21.0$ Hz, 4 H), 2.84 (q, 6H), 1.10 (t, $J = 6.9$ Hz, 9H). $^{31}$P NMR (160 MHz, DMSO-$d_6$, 298 K, TMS): 19.8. ESI MS: Calcd. for $C_{40}H_{39}NO_6P_2$ $[M] = 589.10$, 691.2. Found: 589.50, 689.1. HR-MS calcd for $C_{34}H_{23}O_6P_2$, 589.1000; found, 589.0996.

8. Berova, N.; Nakanishi, K.; Woody, R. W. *Circular Dichroism—Principle and Applications*, 2nd ed.; Wiley-VCH: New York, 2000.

9. (a) Levitus, M.; Garcia-Garibay, M. *J. Phys. Chem. A* **2000**, *104*, 8632; (b) Levitus, M.; Schmieder, K.; Ricks, H.; Shimizu, K. D.; Bunz, U. H. F.; Garcia-Garibay, M. *J. Am. Chem. Soc.* **2001**, *123*, 4259; (c) Wang, Z. X.; Shao, H. X.; Ye, J. C.; Tang, L.; Lu, P. *J. Phys. Chem. B* **2005**, *109*, 19627.

10. (a) Oda, M.; Nothofer, H. G.; Lieser, G.; Scherf, U.; Meskers, S. C. J.; Nether, D. *Adv. Mater.* **2002**, *12*, 362; (b) Lwaura, R.; Hoeben, F. J. M.; Masuda, M.; Schenning, A. P. H. J.; Meijer, E. W.; Shimizu, T. *J. Am. Chem. Soc.* **2006**, *128*, 13298.