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A cationic tetrahedral chromophore for amplified DNA detection

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Dedicated to Professor Guillermo C. Bazan, University of California, Santa Barbara

Abstract—The tetrahedral cationic chromophore, tetrakis [4-(9,9-bis(6'-(N,N,N-trimethylammonium)hexyl)-2-fluorenyl)phenyl]-methane (**1**) shows better fluorescence resonance energy transfer (FRET) to the fluorescein (Fl) attached to the 5'-terminus of double-stranded DNA (dsDNA-Fl) as compared to the linear oligomers **2** and **3** and also provides efficient DNA hybridization detection.
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Advances in DNA sequence identification and detection-based technology allow high-throughput gene expression profiling and nucleotide polymorphism detection, which have profound implications in medical sciences.[1] Although fluorescence-based detection methods are well established, efforts to enhance the sensitivity by developing simplified protocols are still continuing.[2] Water-soluble conjugated polymers have shown large quenching efficiencies in the presence of oppositely charged acceptors and the resulting optical amplification of fluorescent signals has been used for devising strand-specific DNA, RNA and protein detection.[3–6]

In this contribution, we report the synthesis of a novel water-soluble tetrahedral chromophore, tetrakis [4-(9,9-bis(6'-(N,N,N-trimethylammonium)hexyl)-fluorenyl)-phenyl]methane (**1**) (Fig. 1). The key step in the synthesis (Scheme 1) of chromophore **1** is the Pd-catalyzed coupling of 2-bromo-9,9-bis(6'-(N,N-dimethylamino)-hexyl)-fluorene[6] with tetrakis-[4-(4',4',5',5'-tetramethyl-1',3',2'dioxaborolanephenyl)]methane (**5**) to yield[7] tetrakis[4-(9,9-bis(6'-(N,N-dimethylamino)hexyl)-2-fluo-renyl)phenyl]methane (**6**). The quarternization of **6** with excess methyl iodide gave the title compound **1** in good yield.[8] Our interest to develop tetrahedral molecular
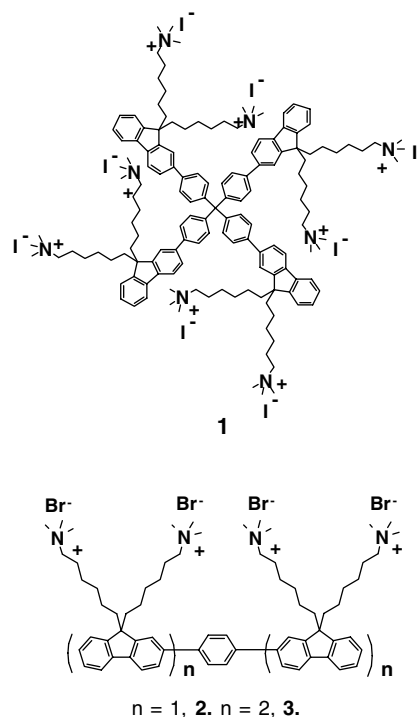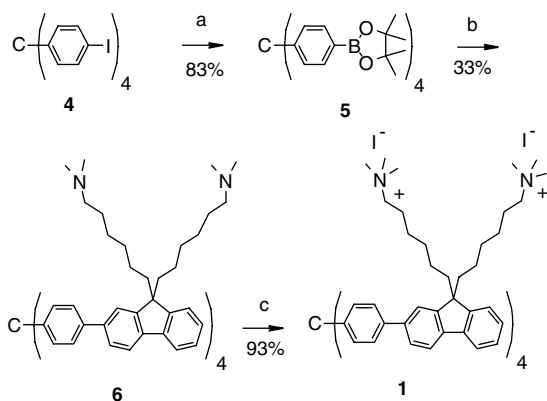

Figure 1. Structures of oligomers **1–3**.

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assemblies stems from the fact that these materials exhibit useful optical and electronic properties and can be purified to a very high degree using conventional organic

Scheme 1. Synthesis of tetrahedral chromophore **1**. Reagents and conditions: (a) Bis(pinacolato)diboron, Pd(dppf)Cl$_2$, KOAc, DMSO, 100 °C; (b) 2-bromo-9,9-bis(6′-(*N*,*N*-dimethylamino)hexyl)fluorene, Pd(PPh$_3$)$_4$, Na$_2$CO$_3$, toluene, water, reflux; (c) Ch$_3$I, THF, water.

Figure 3. The integrated emission intensity of Fl for **1**/dsDNA-Fl (blue line), **2**/dsDNA-Fl (black line) and **3**/dsDNA-Fl (red line) as a function of concentration.

chemistry techniques. Tetrahedral molecules of this type offer distinct advantages over conjugated polymers. They have well-defined structures and relative to their polymer counterparts, there is no statistical distribution of chain lengths and no negative contribution from unreacted end groups. Furthermore, these tetrahedral molecules form stable homogeneous films[9] enabling their use in various optoelectronic and sensor applications. The absorption and fluorescence data for compounds **1**–**3** (Fig. 1) are shown in Figure 2. Both the absorption and emission spectra of **1** are blue shifted compared to those of **2** and **3**. The absorption spectra of fluorescein (Fl) overlaps with the emission spectra of chromophores **1**–**3**, which is necessary for efficient Förster energy transfer between these molecules.[2] Fluorescence quantum efficiencies (QEs) were determined in buffer solution against quinine sulfate in 0.1 M H$_2$SO$_4$ (QE = 57%) as standard. The QE of **1** was 39%, which is lower than those obtained for **2** and **3**[10] (93% and 76%), respectively.

A dsDNA-Fl from the probe having the sequence 5′-AGC ACC CAC ATA GTC AAG AT-3′ with its complimentary ssDNA (5′-AGC ACC CAC ATA GTC AAG AT-3′) was chosen for our experime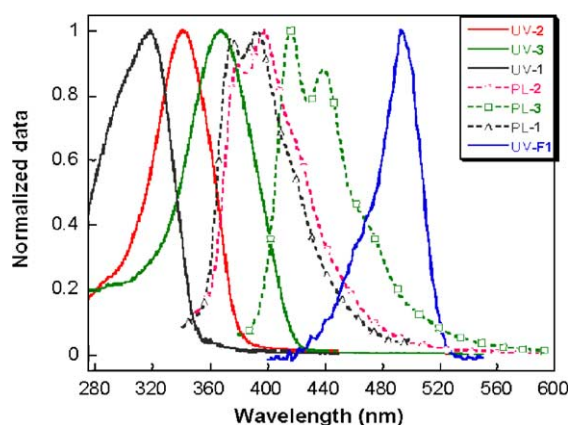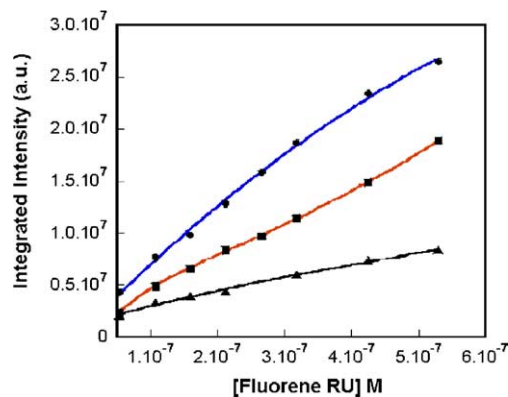nts. The double stranded DNAs were obtained by annealing mixtures of complimentary strands in buffer solution at 57.5 °C (2 °C below its melting point) for 25 min and then cooling to room temperature in phosphate buffer solution (pH = 7.4, 50 mM). Fluorescein was chosen since it is one of the ubiquitous dyes used in FRET experiments and because it can be attached to DNA structures using well-established protocols.[11]

Figure 3 establishes that the interactions of **1**, **2** and **3** with ds-DNA-Fl are size and structure specific and sensitive to the oligomer structure. Buffered conditions were chosen in all our experiments as they stabilize DNA–DNA interactions and would be included in any DNA hybridization assay.[12] The tetrahedral chromophore **1** resulted in better FRET efficiencies with dsDNA-Fl as compared to the linear oligomers **2** and **3**. This important result strongly favors the hypothesis that electrostatic and hydrophobic interactions between negatively charged DNA and cationic chromophores are size and structure specific and are consistent with the literature reports.[13,14]

Since **1** provides better FRET to dsDNA-Fl, we further studied its use for DNA hybridization detection. Figure 4 shows that the FRET to dsDNA-Fl is more efficient relative to the non-complementary DNA (5′-CGT ATC ACT GGA CTG ATT GG-3′). It is known that dsDNA has a larger charge density than ssDNA, thus stronger electrostatic interactions between **1** and dsDNA result in more efficient FRET. In the presence of non-hybridized DNA, there is no formation of dsDNA and the non-complementary ssDNA shows competition for interaction with **1** to ssDNA-Fl and hence the energy transfer is less efficient. The integrated emission intensity of Fl by energy transfer for dsDNA-Fl is three times higher than that for the non-hybridized DNA. This result shows that **1** can be used as a platform for DNA hybridization detection.

In summary, we have reported the synthesis of the new cationic tetrahedral chromophore **1**. Tetrahedral chromophore **1** demonstrates improved FRET to dsDNA-Fl compared to linear oligomers **2** and **3**, which proves that the hydrophobic and electrostatic interactions are size and structure specific. Preferred FRET by chromo-
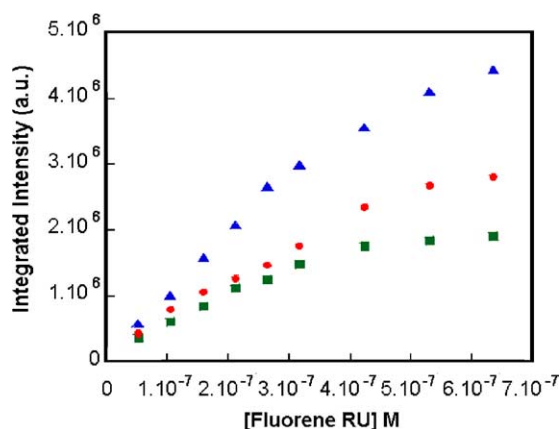

Figure 2. The absorption (UV) and fluorescence (PL) spectra of **1**–**3** in phosphate buffer solution (pH = 7.4, 50 mM).

Figure 4. The integrated emission intensity of Fl for **1**/dsDNA-Fl (blue), **1**/ssDNA-Fl + non-hybridized ssDNA (green) and **1**/ssDNA-Fl (red) as a function of concentration.

phore **1** to ds-DNA-Fl than to ssDNA-Fl + non-hybridized ssDNA and ssDNA-Fl provides an opportunity for using **1** in DNA-hybridization assays. Chromophore **1** presents a simple, homogeneous and sensitive platform for detecting DNA hybridization.

Acknowledgements

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8. Tetrakis[4-(4′,4′,5′,5′tetramethyl-1′,3′,2′dioxaborolane)-phenyl]methane **5**: ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.67 (d, 8H), 7.29 (d, 8H), 1.31 (s, 48H). ¹³C NMR (125 MHz, CDCl₃, ppm): δ 149.56, 134.24, 130.37, 83.82, 65.99, 25.12. MS (Fast atom bombardment-FAB): 824(M). Elemental analysis: calculated for C₄₉H₆₄B₄O₈: C, 71.40; H, 7.83. Found: 70.80, 7.13. Tetrakis[4-(9,9-bis(6′-(N,N-dimethylamino)hexyl)-2-fluorenyl)phenyl]methane **6**: ¹H NMR (400 MHz, CDCl₃, ppm): δ 7.69 (m, 28H), 7.51 (m, 8H), 7.32 (m, 8H), 2.12 (m, 64H), 2.00 (m, 16H), 1.26 (m, 16H), 1.07 (m, 32H), 0.67 (m, 16H). ¹³C NMR (125 MHz, CDCl₃, ppm): δ 151.39, 150.96, 145.92, 140.92, 140.59, 139.48, 139.19, 131.68, 127.20, 126.99, 126.44, 125.99, 122.98, 121.29, 120.13, 119.89, 64.42, 59.95, 55.23, 45.63, 40.55, 30.09, 27.76, 27.25, 23.89. MS (Fast atom bombardment-FAB): 1995(M). Tetrakis[4-(9,9-bis-(6′-(N,N,N-trimethylammonium)hexyl)-2-fluorenyl)phenyl]-methane **1**: ¹H NMR (500 MHz, DMSO-d⁶, 90 °C, ppm): δ 7.85 (m, 24H), 7.45 (m, 20H), 3.38 (m, 32H), 2.96 (m, 72H), 1.47 (m, 16H), 1.13 (m, 32H), 0.65 (m, 16H). ¹³C NMR (125 MHz, DMSO, 90 °C, ppm): δ 151.95, 151.29, 146.42, 141.09, 139.23, 138.97, 138.05, 131.97, 128.15, 127.93, 126.91 126.49, 123.92, 121.67, 121.12, 120.73, 66.75, 64.83, 55.77, 53.54, 31.29, 29.32, 26.13, 24.18, 22.81. MS (TOF-ESI-electrospray ionization): 1438.2 (M-2I), 916.1 (M-3I), 655.4 (M-4I), 499.1 (M-5I), 394.6 (M-6I), 320.2 (M-7I), 265.2 (M-8I).
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