JOC Notes

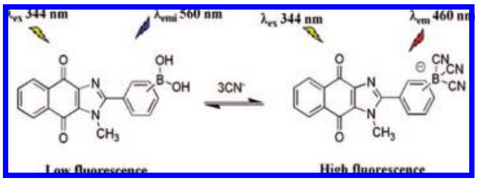
A-D-A Sensors Based on Naphthoimidazoledione and Boronic Acid as Turn-On Cyanide Probes in Water

Matinee Jamkratoke,† Vithaya Ruangpornvisuti,†
Gamolwan Tumcharern,‡ Thawatchai Tuntulani,† and
Boosayarat Tomapatanaget†,*

Supramolecular Chemistry Research Unit, Department of Chemistry, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand, and National Nanotechnology Center, National Science and Technology Development Agency, Patumthanee 12120, Thailand

tboosayarat@gmail.com

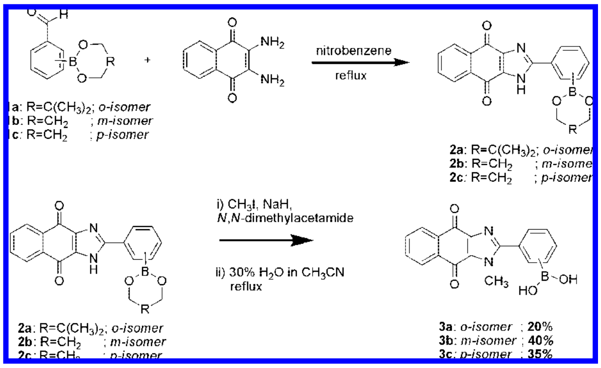
Received January 26, 2009



Three fluorescence sensors based on naphthoquinoneimidazole and boronic acid (A-D-A system) have been developed with high selectivity for cyanide in water. The fluorescence band at 460 nm was switched on upon substitution of cyanide on sensors in the CTAB micelle.

Cyanide ion is one of the analytes of most concern in environments due to its toxicity. The toxicity of the cyanide ion is attributed to its ability to effectively bind with the active site of cytochrome *c* oxidase resulting in the disruption of the electron transport chain. Tissues that mainly depend on aerobic respiration, such as the central nervous system and the heart, are acutely affected.[1] Fluorescent detection of CN$^-$ is one of the best tools because of its excellent sensitivity. Several organic and inorganic compounds such as boronic acid,[2] cationic boran,[3] oxazine,[4] squarine,[5] dipyrrole carboxamide,[6] acridinium salt,[7] acyltriazenes,[8] and coumarin[9]derivatives were used as sensors for detecting cyanide employing the nucleophilic substitution reaction.

SCHEME 1. Synthesis Pathway of 3a, 3b, and 3c



From a variety of signal transductions, many researchers are interested in taking the profit of the internal charge transfer (ICT) process, which is well-known to be extremely sensitive to small perturbations.[10] Usually, common ICT organic dyes consist of electron acceptor and electron donor sites.[11] To be a chemosensor, the interaction of a guest molecule (cation or anion) with an electron donor or an acceptor site must induce the fluorescence or absorption changes. Recently, chemists have paid attention to boronic acid based sensors for detecting the cyanide ion. Although there were many reports regarding highly sensitive chemosensors for the cyanide ion, a few sensors gave a large wavelength shift and high intensity change in their emission spectra.[2] It is a challenging task to design turn-on cyanide probes which can perform a fluorescence intensified signal with a large shift. Therefore, we have developed high response fluorescence probes using an acceptor–donor–acceptor (A-D-A) system. The A-D-A sensors are composed of naphthoquinone connecting to boronic acid with imidazole as a conjugative spacer. Napthoquinone and imidazole groups are a main A-D system that is expected to have a large dipole moment change when excited

---

† Chulalongkorn University.

‡ National Science and Technology Development Agency.

(1) (a) Hathaway, G. J.; Proctor, N. H. *Chemical Hazards of the Workplace*, 5th ed.; John Wiley & Sons, Inc: Hoboken, NJ, 2004; pp 190−191. (b) Patnaik, P. *A Comprehensive Guide to the Hazardous Properties of Chemical Substance*; van Nostrand Reinhold: New York, 1992; pp 229−244. (c) Ishii, A.; Seno, H.; Watanabe-Suzuki, K.; Suzuki, O.; Kumazawa, T. *Anal. Chem.* **1998**, *70*, 4873–4876.

(2) (a) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *Curr. Anal. Chem.* **2005**, *1*, 157–170. (b) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *Dyes Pigments* **2005**, *64*, 49–55. (c) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *Anal. Biochem.* **2004**, *327*, 82–90. (d) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *J. Am. Chem. Soc.* **2005**, *127*, 3635–3641. (e) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *Anal. Chem. Acta* **2004**, *522*, 9–17.

(3) Hudnall, T. W.; Gabbai, F. P. *J. Am. Chem. Soc.* **2007**, *129*, 11978–11986.

(4) (a) Tomosulo, M.; Raymo, F. M. *Org. Lett.* **2005**, *7*, 4633–4636. (b) Tomosulo, M.; Sortino, S.; White, A. J. P; Raymo, F. M. *J. Org. Chem.* **2006**, *71*, 744–753. (c) Ren, J.; Zhu, W.; Tian, H. *Talanta* **2008**, *75*, 760–764.

(5) Ros-Lis, J. V.; Martines-Manes, R.; Soto, J. *Chem. Commun.* **2002**, 2248–2249.

(6) Chen, C.-L.; Chen, Y.-H.; Chen, C.-Y.; Sun, S.-S *Org. Lett.* **2006**, *8*, 5053–5056.

(7) Yang, Y.-K.; Tae, J. *Org. Lett.* **2006**, *8*, 5721–5723.

(8) Chung, Y.; Lee, H.; Ahn, K. H. *J. Org. Chem.* **2006**, *71*, 9470–9474.

(9) Lee, K.-S.; Kim, H.-J.; Shin, I.; Hong, J.-I. *Org. Lett.* **2008**, *10*, 49–51.

(10) Valure, B. *Topic in Fluorescence Spectroscopy*; Lakowizc, J. R., Ed.; Plenum: New York, 1994.

(11) (a) Fery-Forgues, S.; Le Bris, M.-T.; Guette, J.-P.; Valuer, B. *J. Phys. Chem. A* **1988**, *92*, 6233–6237. (b) Fery-Forgues, S.; Le Bris, M.-T.; Mialocq, J.-C.; Pouget, J.; Rittig, W.; Valuer, B. *J. Phys. Chem. A* **1992**, *96*, 701–710. (c) Marcotte, N.; Plaza, P.; Lavabre, D.; Fery-Forgues, S.; Martin, M. M. *J. Phys. Chem. A* **2003**, *107*, 2394–2402.

10.1021/jo900170r CCC: $40.75

by light.[11,12] These sensors utilize an alternation of electron deficiency to electron rich of the boron center to give a distinct change in their spectra. This process is stimulated by cyanide substitution on the boron atom.

The synthetic pathway of new fluorescent sensors **3a**, **3b**, and **3c** is shown in Scheme 1. The oxidative condensation[13] of 2,3-diamino-1,4-naphthoquinone[14] and the protected corresponding formylphenylboronate in nitrobenzene yielded the precipitate of heterocyclic compounds **2a**, **2b**, or **2c** in 29%, 45%, and 71% yield, respectively. These products were indicated by the appearance of the NH proton at ca. 14.3 ppm and the new series of aromatic protons at around 8.09 and 7.84 ppm in the ^{1}H NMR spectra. Methylation on the N-atom of the heterocyclic ring was achieved by the reaction of the protected derivative with CH_3I in the presence of NaH in *N*,*N*-dimethylacetamide at room temperature. Finally, the ester protecting group was removed by refluxing in 30% H_2O:CH_3CN to give **3a**, **3b**, and **3c** as yellow solids in 20%, 40%, and 45% yields, respectively.

In the mixture of DMSO:H_2O (50 μM of receptor in 0.1 mol/L of NaCl in 50% HEPES pH 7.4:DMSO), sensors **3a**, **3b**, and **3c** gave yellow solutions with absorption maxima at 338 and 387 nm. Moreover, all sensors showed the characteristic luminescence with different emission maxima at 562, 565, and 572 nm for ortho, meta, and para isomers, respectively. Figure 1a shows fluorescence spectra of compound **3b** with and without 500 equiv of anions. A high-intensity fluorescence band at 460 nm was observed upon addition of cyanide into solutions of sensor **3b**. The high response was also observed in sensor **3c**, whereas the ortho isomer, **3a**, showed a slight response in the presence of CN^- (Figure 1b). Furthermore, upon exposure to UV irradiation at 365 nm, the solution of **3b** + CN^- gave a brighter luminescence than that of **3b** + F^- as shown in Figure 2.

In the presence of a very high concentration of cyanide ion (500 equiv), pH 7.4 HEPES solution was changed to pH 11. It is possible that at high pH, OH^- could be generated and substituted on the boron center to form $RB(OH)_3^-$. This weak point was averted by incorporating compounds **3b** and **3c** to a CTAB micellar system to allow the detection of CN^- at vary low concentration (50 μM). In this system, the pH of the solution remained pH 7.0 and the emission band at 460 nm appeared intensely upon the addition of 50 μM CN^- as shown in Figure 3 as well as Figure S9 in the Supporting Information.

Moreover, the probe **3b** in the CTAB micellar system exhibited promising selective binding with cyanide ion similar to that in 50% HEPES pH 7.4:DMSO. This suggested that the boron center was substituted with the cyanide anion. The hybridization of the boron center changed from the electron deficiency sp^2 boron, R-$B(OH)_2$, to the electron rich sp^3 boron, R-$B(CN)_3$,[15,16] resulting in the emission band at 460 nm.

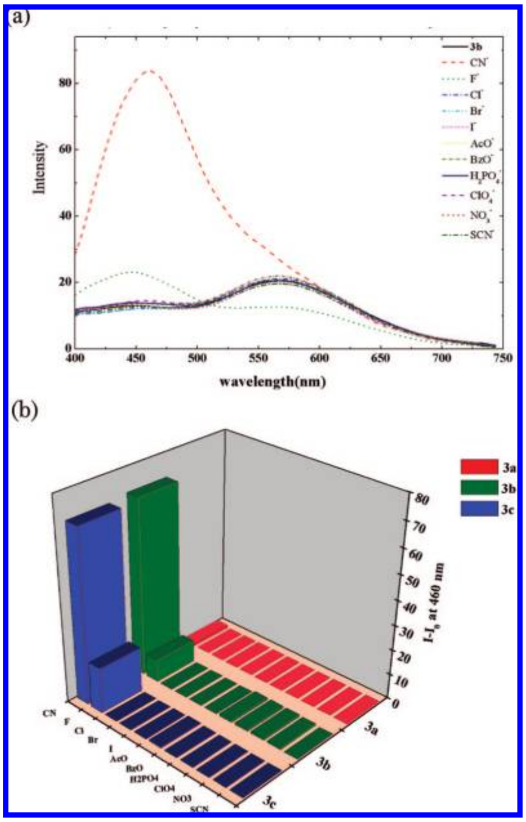


FIGURE 1. (a) Fluorescence spectral changes of **3b** after the addition of 500 equiv of various anions (potassium salts). (b) Fluorescence responses of **3a**, **3b**, and **3c** with 500 equiv of various anions (50 μM of receptor in 0.1 mol/L of NaCl in 50% HEPES pH 7.4:DMSO).

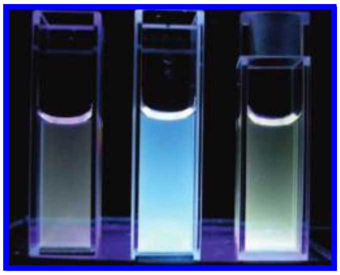


FIGURE 2. Fluorescence responses of 50% HEPES pH 7.4:DMSO solutions of **3b** (left), **3b** + CN^- (middle), and **3b** + F^- (right).

Fluorescence responses of sensors **3b** and **3c** in the presence of 50 μM of CN^- showed a large response giving a high intensity change (18-fold enhancement) and a large stoke shift ($\Delta\lambda_{\text{ex-em}} = 120$ nm) as well as a large blue shift of ca. 100 nm. The fluorescence band at 460 nm possibly assigned to the ICT state was switched on by the cyanide ion. This ICT state was predominant with a poor acceptability on the boron center after cyanide addition. A large fluorescence response toward CN^- recognition of these receptors occurred since the boronic center behaved as a complementary acceptor of a standard electron donor−acceptor system (D-A or A-D).[11] The presence of the electron donor (imidazole group) and the electron acceptor (naphthoquinone group) as a main A-D site caused a prerequisite dipole moment change resulting in a large change in spectral properties.[11]

For better understanding of the fluorescence behaviors, structures of the sensors **3a**−**c** and their cyanide-substituted products have been calculated by using density function theory (DFT) at the B3LYP/6-31+G(d) level. Natural bond orbital (NBO) charges of donor−acceptor segments of sensors

---

[12] de Silva, A. P.; Gunaratne, H. Q. N.; Habib-Jiwan, J. L.; McCoy, C. P.; Rice, T. E.; Soumillion, J. P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1728–1731.

[13] Kim, S. J.; Kool, E. T. *J. Am. Chem. Soc.* **2006**, *128*, 6164–6171.

[14] Wunkelmann, E. *Tetrahedron* **1969**, *25*, 2427–2454.

[15] (a) Lorand, J. P.; Edward, J. O. *J. Org. Chem.* **1959**, *24*, 769–774. (b) Katz, H. E *J. Am. Chem. Soc.* **1986**, *108*, 7640–7645.

[16] (a) Cooper, C. R.; Spencer, N.; James, T. D. *Chem. Commun.* **1998**, 1365–1366. (b) DiCesare, N.; Lakowizc, J. R. *Anal. Biochem.* **2002**, *301*, 111–116. (c) Kubo, Y.; Kobayashi, A.; Ishida, T.; Misawa, Y.; James, T. D. *Chem. Commun.* **1998**, 1365–1366. (d) Badugu, R.; Lakowizc, J. R.; Geddes, C. D. *Sens. Actuators.* **2005**, *104*, 103–110.

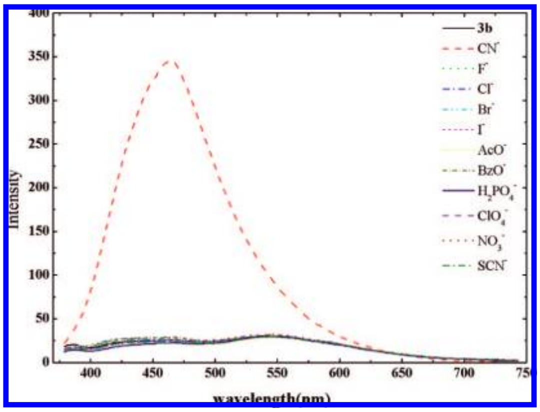
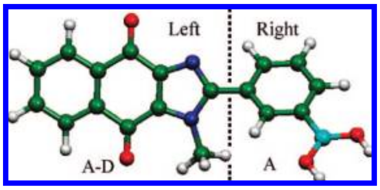


FIGURE 3. Fluorescence spectra of **3b** (50 μM) with 50 μM of various anions in the CTAB micellar system (5 mM of CTAB in 10% ethanol: water).

TABLE 1. NBO Charges of Segments of 3a, 3b, and 3c and of 3a(CN)$_3^-$, 3b(CN)$_3^-$, and 3b(CN)$_3^-$ Derived from the B3LYP/ 6-31+G(d) Computations



species	charge^a left segment	right segment	total charge^a
free hosts			
3a	−0.02685	0.02685	0.00
3b	−0.03985	0.03985	0.00
3c	−0.02358	0.02358	0.00
full CN-substituted hosts			
3a(CN)$_3^-$	−0.04235	−0.95762	−1.00
3b(CN)$_3^-$	−0.08330	−0.91670	−1.00
3c(CN)$_3^-$	−0.01968	−0.97892	−1.00

[a] The elementary charge unit = 1.602 × 10^{-19} coulombs.

3a−c suggested that napthoquinoneimidazole possessing negative charges acted as a donor site and boronic acid possessing positive charges acted as an acceptor site (Table 1). Upon the addition of CN$^-$, the right segment charge became negative corresponding to the reduction in acceptability of boronic acid and subsequent appearance of a fluorescence band at 460 nm.

Considering the sensitivity of each isomer, calculated structures (Figure S10, Supporting Information) showed that the dihedral angles of the donor and acceptor planes of **3a**, **3b**, and **3c** were 56.5°, 37.5°, and 37.7°, respectively. After cyanide substitution, the dihedral angles of CN-substituted **3b** and **3c** changed slightly (~2°) while that of substituted **3a** changed significantly (nearly 12°). This probably inhibited the substitution of cyanide in the boronic center of **3a.** The calculated structures thus agreed very well with the experimental results, which showed that the emission spectra of **3a** before and after cyanide substitution were insignificantly different.

In summary, we have successfully synthesized A-D-A fluorescence sensors containing naphthoquinoneimidazole and boronic acid, **3a**, **3b**, and **3c**, as turn-on cyanide probes in the CTAB micellar system. In this approach, CTAB can incorporate **3b** and **3c** to provide powerful probes for detecting a very low concentration of CN$^-$ in water. These A-D-A probes offer considerable promises as cyanide selective fluorescence probes with a large response emission band at 460 nm and a large blue shift (ca. 100 nm).

Experimental Section

Compounds 2a, 2b, and 2c. The protected formylphenylboronate was synthesized according to literature procedure.[17] To a solution of 2,3-diamino-1,4-naphthaquinone (0.940 g, 5 mmol) in nitrobenzene (75 mL) was added the protected corresponding formylphenylboronate (5 mmol) in nitrobenzene (25 mL) dropwise. The reaction mixture was heated at 150 ^{o}C and stirred for 12 h under nitrogen. The solution was then cooled to room temperature and the precipitate was slowly formed. The precipitate was filtered and washed with diethyl ether to give a yellow solid of the protecting products (**2a** 29%, **2b** 45%, and **2c** 71%).

Compound 2a: ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.40 (broad, 1H), 8.12 (m, 3H), 7.84 (m, 2H), 7.47 (m, 3H), 3.74 (s, 4H), 1.06 (s, 6H). Anal. Calcd for $C_{22}H_{19}BN_2O_4$: C, 68.42; H, 4.96; N, 7.25. Found: C, 67.99; H, 4.82; N, 7.55. EI-MS *m/z* 387.15 (M + 2H)$^+$.

Compound 2b: ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.42 (s, 1H), 8.57 (s, 1H), 8.26 (d, J = 7.6 Hz, 1H), 8.083 (m, 2H), 7.83 (m, 2H), 7.77 (d, J = 7.6 Hz, 1H), 7.50 (t, J = 7.2 Hz, 1H), 4.14 (t, J = 4.8 Hz, 4H), 2.02 (t, J = 5.2 Hz, 2H). Anal. Calcd for $C_{22}H_{19}BN_2O_4$: C, 67.07; H, 4.22; N, 7.82. Found: C, 66.96; H, 4.23; N, 7.88. MALDI-TOF *m/z* 359.64 (M + 2H)$^+$.

Compound 2c: ^{1}H NMR (400 MHz, DMSO-d_6) δ 14.39 (s, 1H), 8.19 (d, J = 8.5 Hz, 2H), 8.09 (m, 2H), 7.49 (m, 2H), 7.78 (d, J = 8.0 Hz, 2H), 4.12 (t, J = 4.1 Hz, 4H), 2.01 (t, J = 5.2 Hz, 2H). Anal. Calcd for $C_{20}H_{15}BN_2O_4$: C, 67.07; H, 4.22; B, 3.02; N, 7.82. Found: C, 67.06; H, 4.59; N, 7.76. MALDI-TOF *m/z* 358.63 (M + H)$^+$.

Compounds 3a, 3b, and 3c. Compounds **2a**, **2b**, or **2c** (5 mmol) and NaH (0.132 g, 5.5 mmol) were charged with 25 mL of *N*,*N*-dimethylacetamide under nitrogen. Methyl iodide (343 μL, 5.5 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 2 days. The solvent was removed under vacuum to give the solid of methylated products. The protecting group was removed by refluxing in 30% H_2O: CH_3CN. The solution was filtered and washed with diethyl ether to provide a yellow solid of compound **3** (**3a** 20%, **3b** 40%, and **3c** 35%).

Compound 3a: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (m, 2H), 8.01 (s, 2H), 7.87 (m, 2H), 7.77 (m, 1H), 7.54 (m, 3H), 3.84 (s, 3H). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 178.9, 174.4, 157.0, 142.6, 134.6, 134.5, 134.4, 133.1, 132.7, 129.8, 129.6, 126.8, 126.6, 34.3. Anal. Calcd for $C_{18}H_{23}BN_2O_4$: 65.10; H, 3.95; N, 8.43. Found: C, 65.27; H, 3.98; N, 8.48. MALDI-TOF *m/z* 334.66 (M + 3H)$^+$.

Compound 3b: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.29 (s, 2H), 8.21 (s, 1H), 8.09 (t, J = 8.1 Hz, 2H), 7.99 (d, J = 8.0 Hz, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.56 (t, J = 7.5 Hz, 1H), 4.06 (s, 3H). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 178.9, 176.4, 154.9, 143.0, 136.5, 135.5, 134.5, 134.3, 133.8, 133.3,132.9, 131.3, 128.3, 127.8, 126.8, 126.6, 34.8. Anal. Calcd for $C_{18}H_{23}BN_2O_4$: 65.10; H, 3.95; N, 8.43. Found: C, 65.28; H, 3.90; N, 8.48. MALDI-TOF *m/z* 331.60 (M)$^+$.

Compound 3c: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.26 (s, 2H), 8.09 (t, J = 8.0 Hz, 2H), 7.98 (d, J = 8.0 Hz, 2H), 7.86 (t, J = 7.8 Hz, 2H), 7.78 (d, J = 7.8 Hz, 2H), 4.08 (s, 3H). ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 178.8, 176.4, 154.5, 143.0, 134.7, 134.5, 134.3, 133.8, 133.3, 132.8, 129.9, 128.7, 126.8, 126.6, 34.8. Anal. Calcd for $C_{18}H_{23}BN_2O_4$: 65.10; H, 3.95; N, 8.43. Found: C, 65.07; H, 3.90; N, 8.56. MALDI-TOF *m/z* 332.39 (M + H)$^+$.

---

(17) James, T. D.; Sandanayake, K. R. S.; Iguchi, R.; Shinkai, S. *J. Am. Chem. Soc.* **1995**, *117*, 8982–8987.

Fluorescence Measurement Procedure of Sensors 3a−3c with Excess Anions in 50% HEPES pH 7.4:DMSO. Receptors **3a**, **3b**, and **3c** were prepared as stock solution in spectroscopic grade DMSO at concentrations of 0.1 mM. Stock solutions of sensors (1.5 mL) and 500 equiv of anions (KCN, KF, KAcO, KBzO, KH_2PO_4, KNO_3, $KClO_4$, KCl, KBr, KSCN, and KI) which were dissolved in 1.5 mL of 0.2 mol/L of NaCl in HEPES buffer pH 7.4 were mixed to give a final concentration of sensors (50 μM) with 500 equiv of anions added in 0.1 mol/L of NaCl in 50% HEPES pH 7.4:DMSO. Fluorescence spectra were recorded with the excitation wavelength of 344 nm. In the case of the addition of KF, stock solution sensors and KF were mixed to give a final concentration of sensors (50 μM) with 500 equiv of anions added in 0.1 mol/L of NaCl in 60% HEPES pH 7.4:DMSO because of KF solubility.

Fluorescence Measurement Procedure of Sensors 3b with Excess Anion in the CTAB Micellar System. In a 5 mL volumetric flask, 1.0 mL of an ethanol solution of fluorescence probes (0.025 mM) was mixed with 2.0 mL of 12.5 mM CTAB in water. Then, 10 μL of 2.5 mM potassium salt solution (KCN, KF, KAcO, KBzO, KH_2PO_4, KNO_3, $KClO_4$, KCl, KBr, KSCN, and KI) was added to the probe. Fluorescence spectra were recorded with the excitation wavelength of 344 nm.

Acknowledgment. M.J. is a Ph.D student supported by the Royal Golden Jubilee Program (PHD/0049/2550). We thank the National Nanotechnology Center (NN-B-22-b15-94-49-55), the Thailand Research Fund (RTA5080006), and the National Center of Excellence for Petroleum, Petrochemical, and Advanced Materials (NCE-PPAM) for financial support.

Supporting Information Available: Details of the procedure for the synthesis of protected formylphenylboronate; ^{1}H and ^{13}C NMR spectra of **3a**, **3b**, and **3c**; fluorescence spectra changes of **3a**, **3b**, and **3c** after addition of various anions (potassium salts); and other data. This material is available free of charge via the Internet at http://pubs.acs.org.

JO900170R