Article
DOI: 10.1002/bkcs.12163 Y. K. Kim et al. BULLETIN OF THE KOREAN CHEMICAL SOCIETY

Polydiacetylenes Containing 2-Picolylamide Chemosensor for Colorimetric Detection of Cadmium Ions

Yong Kyun Kim,[†] Thanh Chung Pham,[†] Jaewon Kim,[‡] Chaeeon Bae,[†] Yeonghwan Choi,[†] Min Hee Jo,[‡] and Songyi Lee[†,‡,*]

[†]*Interdisciplinary Program of Biomedical, Mechanical and Electrical Engineering, Pukyong National University, Busan 48513, South Korea*
[‡]*Department of Chemistry, Pukyong National University, Busan 48513, South Korea.*
**E-mail: slee@pknu.ac.kr*
Received September 25, 2020, Accepted November 19, 2020

This article describes the preparation and analysis of Cd^{2+}-sensing polydiacetylene-based chemosensors (PDA-MP). Polydiacetylenes (PDAs), a family of conjugated polymers, exhibit a strong response to external stimuli. Accordingly, we have designed a novel PDA sensor, which is linked to mono 2-picolylamine, to increase the PCDA-MP monomer content in polymer system. As a result, it enhanced sensitivity toward Cd^{2+} with the low detection limit (2.10 μM) in aqueous solution. Our sequential modification of chelate structure improved the Cd^{2+}-sensing ability of PDAs. Finally, the interaction between PCDA–MP and Cd^{2+} was confirmed by Raman spectroscopy, Field Emission Scanning Electron Microscopes (FE-SEM), and Proton nuclear magnetic resonance (^{1}H NMR).

Keywords: Polydiacetylenes, PDAs sensors, Colorimetric sensor, Fluorescent sensor, Cadmium sensor

Introduction

Heavy metals are important to maintain multiple biochemical and metabolic activities, but exposure to excessive concentrations represents an increasingly serious threat.[1] Besides, they lead to significant environmental contamination and accumulate in the human body, resulting in serious diseases.[2] Among the different heavy metals, cadmium is one of the most dangerous and hazardous elements. The absorption and accumulation by organisms cause severe diseases and even cancer.[3] For that reason, various analytical methods have been developed to detect Cd^{2+} using fluorescent and colorimetric sensors.[4] However, the selective recognition of cadmium compared with zinc and mercury ions is a challenge because of their closed-shell d^{10} electronic configurations.[5] In this regard, we developed highly selective and sensitive chemosensor based on polydiacetlyenes (PDAs) for visual detection of Cd^{2+}.

PDAs have attracted great attention as intelligent materials due to their fascinating properties.[6] PDAs can be polymerized from self-assembled diacetylene monomers by UV[7] or plasma irradiation.[8] In general, PDAs show a non-fluorescent "blue phase," which undergoes dramatic color transition from blue to red (λmax ≈ 640–550 nm) by various stimuli, resulting in fluorescent enhancement.[9]

In a previous study, we prepared PCDA-CP, a diacetylene monomer consisting of chelidamic acid and 2-picolylamine, which recognize Cd^{2+} under specific conditions with comparable low detection limit.[10] We developed another diacetylene monomer (PCDA-HP) with remarkable selectivity.[11] The PCDA-HP monomer was deficient in a single nitrogen compared to PCDA-CP and decreased the chelating ability with another metal (Figure 1.). However, the attempt to convert to PDA polymer using only PCDA-HP monomers failed, due to the steric hindrance of the bulky head group. Finally, PDA-HP was prepared with a low content of chelating HP moiety (PCDA-HP and PCDA in a ratio of 1:9). Given that the chelate content depends on the number of detectable analytes, increasing the ratio of monomers linked to chelate moiety can facilitate the detection of target molecules with high efficiency. Thus, we have introduced a small head group linked to a mono 2-picolylamine (MP) moiety, which still retains the key structure for Cd^{2+} chelation. The PDA-MP solutions consisting of PCDA-MP and PCDA at a 5:1 ratio with a high content of chelate display excellent color transition toward Cd^{2+}.

Experimental

Materials. Unless otherwise noted, materials were obtained from commercial suppliers and were used without further purification. Flash chromatography was carried out on silica gel (230–400 mesh) followed by determination of ^{1}H and ^{13}C NMR spectra using a Bruker Avance 400 MHz NMR spectrometer. Chemical shifts were expressed in ppm, and coupling constants (*J*) in Hz. Mass spectra were obtained using a maXis-HD (Bruker, Billerica, MA). UV absorption spectroscopy measurements were carried out on V-730 UV–Visible spectrophotometer (Jasco) at room temperature. Fluorescence emission spectra were obtained

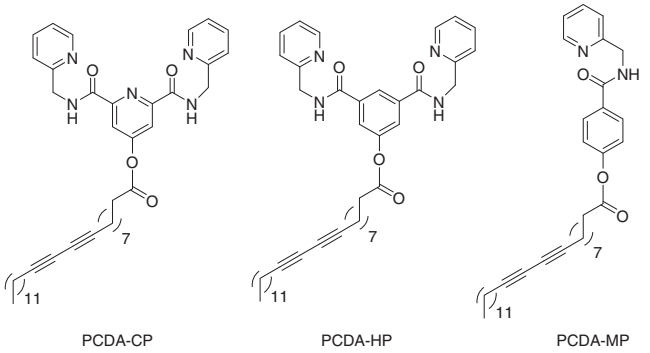

Figure 1. Structures of PDA monomers (PCDA-CP, PCDA-HP, and PCDA-MP) for Cd^{2+} sensing.

using an F-7000 Fluorescence Spectrophotometer (Hitachi High-Tech, Tokyo, Japan).

Preparation of PDA Micelle. The PDA micelle were formed using ultrasonication at high temperature. A mixture of PCDA-MP and PCDA with 5:1 ratio was dissolved in Dichloromethane (DCM) (10 mL). The mixture was stirred and heated with a stream of nitrogen gas. After organic solvent was dried, D.W (20 mL) was added to mixture. The resulting solution was ultrasonicated for 30 min at 80 °C. And then solution including micelle was kept at 0 °C overnight. PDA micelles were prepared by illumination of 254 nm UV for 18 min, presenting a blue-colored solution.

Synthesis of 4-hydroxy-*N*-(pyridin-2-ylmethyl) benzamide (MP). Oxalyl chloride (1 mL, 11.45 mmol) was added dropwise to a THF (20 mL) solution containing 4-hydroxybenzoic acid (0.5 g, 3.62 mmol) under N_2 at room temperature. After 1 h of stirring, Dimethylformamide (DMF) (0.2 mL) was added to the solution and the resulting mixture was stirred during 4 h, followed by concentration under reduced pressure to yield diacyl chloride as a yellow solid, which was used directly without purification by dissolving in Acetonitrile (ACN) (20 mL). It was added to 10 mL ACN containing 2-picolylamine (0.53 mL, 1.5 eq). The reaction mixture was stirred and refluxed overnight under N_2. This mixture was purified by silica gel column chromatography (DCM: MeOH = 20: 1) as eluent to obtain a white solid (yield:49%). Proton nuclear magnetic resonance (^{1}HNMR) (400 MHz, D_2O) δ 8.69–8.67 (dd, J = 6, 0.4 Hz, 1H), 8.55–8.51(td, J = 8, 0.4 Hz, 1H), 8.00–7.98 (d, J = 8 Hz, 1H), 7.96–7.92 (m, 1H), 7.79–7.75 (dt, J = 8.8, 2.8 Hz, 2H), 6.98–6.95 (dt, J = 8.8, 2.8 Hz, 2H), 4.91 (s, 2H) ^{13}C NMR δ (400, D_2O MHz) 170.82, 159.87, 153.22, 147.11, 141.05, 129.76, 125.77, 125.59, 124.02, 115.54, 41.32. ESI HRMS m/z = 227.0824 $[M + H]^+$, calc. for $C_{13}H_{12}N_2O_2$ = 228.25.

Synthesis of 4-((pyridin-2-ylmethyl)carbamoyl)phenyl pentacosa-10,12-diynoate (PCDA-MP). Oxalyl chloride (1 mL, 11.45 mmol) was added dropwise to a DCM (20 mL) solution containing 10,12-pentacosadiynoic acid (PCDA) (0.164 g, 0.84 mmol) under N_2 at room temperature. After 1 h of stirring, DMF (0.1 mL) was added to the solution. The resulting mixture was stirred for 4–6 h, followed by concentration under reduced pressure to obtain PCDA-Cl, which was used directly without purification by dissolving in ACN (15 mL). It was added to MP in ACN (15 mL) and trimethylamine (0.4 mL) was added to solution. The reaction mixture was stirred and refluxed overnight under N_2. This mixture was purified by silica gel column chromatography (Hexane:EA = 1: 2) as eluent to obtain a white solid (yield: 30%). ^{1}HNMR (400 MHz, D_2O) δ 8.57–8.55 (dq, J = 4.8, 0.8 Hz, 1H), 7.92–7.89 (td, J = 8.8, 2 Hz, 2H), 7.72–7.67 (td, J = 7.6,2 Hz, 1H), 7.66–7.63 (t, J = 4.4, 1H), 7.35–7.33 (d, J = 7.6, 1H), 7.25–7.21 (m, 1H), 7.19–7.15 (td, J = 8.8, 2 Hz, 2H), 4.76–4.75 (d, J = 5.2, 2H), 2.59–2.55 (t, J = 7.6, 2H), 2.27–2.22 (q, J = 6.8, 4H), 1.79–1.71 (q, J = 7.6, 2H), 1.56–1.47 (m, 4H), 1.43–1.31 (m, 26H), 0.89–0.86 (t, J = 6.4, 3H), ^{13}C NMR δ (400, D_2O MHz) 157.85, 155.93, 155.93, 150.29, 137.48, 135.05, 131.75, 131.36, 115.10, 115.10, 113.17, 113.17, 96.71, 77.43, 77.31, 77.11, 76.79, 53.53, 46.40, 46.40, 31.70, 22.77, 22.77, 14.24, 12.80, 12.80. ESI HRMS m/z = 585.4051 $[M + H]^+$, calc. for $C_{13}H_{12}N_2O_2$ = 584.85.

Colorimetric Response (CR%) Value Measurement. To quantify degree of color transition, CR% value was calculated according to the following equation:[12]

$$CR = \frac{PB_0 - PB_1}{PB_0} \times 100$$

where $PB = A_{blue}/(A_{blue} + A_{red(violet)})$, A means the absorbance at the PDA of blue (648 nm) or the red (547 nm) color in UV/Vis spectrum. PB_0 is the ratio of the A at 648 nm to that at 547 nm in the absence of Cd^{2+}, while PB_1 is the ratio of the A at 648 nm to that at 537 nm when various concentrations of Cd^{2+} were dealt.

Results and Discussion

Preparation of PDA-MP. The PCDA-MP monomers were difficult to form PDA micelles due to the steric hindrance of head group. We have optimized the appropriate ratio via a series of experiments. PCDA-MP and PCDA formed vesicles at a 5:1 ratio from assembled structures with relatively hydrophilic head group and hydrophobic aliphatic chain. The PDA solutions were prepared by UV irradiation (254 nm, 1 mW/cm^2, 18 min) (Figure 2(a)). The PDA-MP existed as a rigid π-conjugated network along the PDA polymer backbone in a blue-colored solution. The addition of cadmium ion to PDA-MP solution resulted in twisting of the well-aligned backbone structure. As a result, the maxima absorption band shift occurred with a dramatic color transition (Figure 2(b)).

Characterization of PDA-MP Liposomes. To confirm structural change following the addition of cadmium ion,

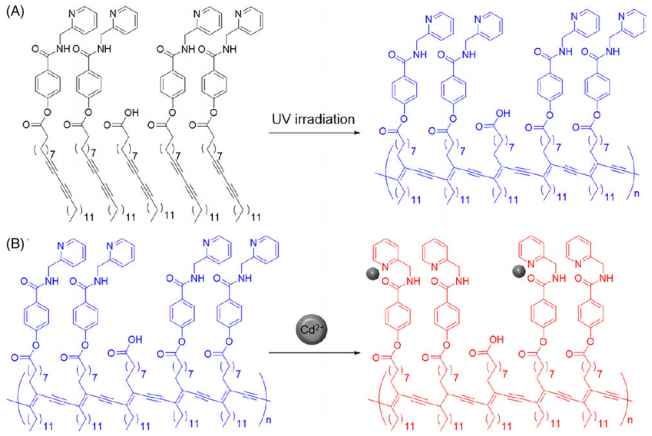

Figure 2. (a) Self-assembly and polymerization of PCDA-MP and PCDA with 5:1 ratio; (b) schematic illustration of PDA colorimetric transition by detection of cadmium ion.

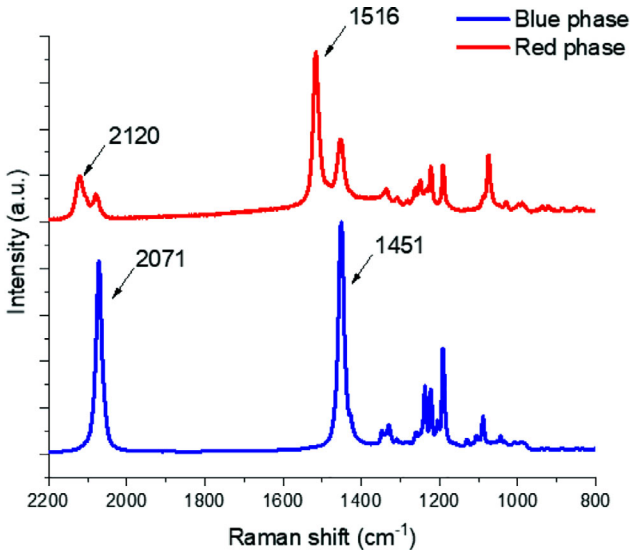

Figure 3. Raman spectra of PDA-MP liposomes at room emperature (blue line) and after treatment with Cd^{2+} (red line).

PDA-MP lioposomes were characterized by Raman spectroscopy, Field Emission Scanning Electron Microscopes (FE-SEM). Raman spectra were analyzed before and after the addition of cadmium ion at room temperature (Figure 3). The C=C and C≡C bond stretching of blue-phase PDAs appeared at 1451 and 2071 cm^{-1}. Compared to double bonds, additional strong triple bonds appeared at a higher energy peak (2071 cm^{-1}). Treatment with Cd^{2+} decreased the original peaks and shift to 151p6 and 2120 cm^{-1}. The chemical shifts suggested that the rigid π-conjugated network was twisted after the addition of cadmium ion, which is a red phase.[13] SEM images of PDA-MP showed structureless particles. However, the treatment with cadmium ions resulted in uniform nano structures due to the formation of metal complexes (Supporting Information Figure S7).

Cadmium Ion Detection Using PDA-MP Micelles. Figure 4(a) displays the selectivity of PDA-MP toward Cd^{2+} in aqueous solutions. The PDA-MP (500 μM)-conjugated polymer sensor turned red using perchlorate (ClO_4^-) as the counter anion in the presence of 1 eq. of Ca^{2+} except other metal ions such as Li^+, Na^+, K^+, Cs^+, Mg^{2+}, Ca^{2+}, Cr^{3+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Ag^+, Zn^{2+}, Cd^{2+}, and Hg^{2+}. This phenomenon also suggests that PDA-MP polymer can be used to easily detect cadmium ion with the naked eye. In Figure 4(b), PDA-MP showed a gradual color transition by increasing the amount of cadmium ions and the resulting PDA-MP showed dramatic color differences between blue and red.

Spectroscopic Properties of PDA-MP in the Presence of Cadmium Ion. The Cd^{2+}-triggered colorimetric and fluorometric changes of PDA-MP were also monitored by UV–vis and fluorescence spectroscopy. The effect of cadmium ion on the absorption spectra of PDA-MP is depicted in Figure 5(a). When the amount of Cd^{2+} was gradually increased, a 547 nm peak emerged as the original peak (648 nm) decreased. As shown in Figure 5(b), the enhanced fluorescent emission intensity following excitation at 540 nm was followed by increasing the concentration of Cd^{2+}. Compared with other metal ions, cadmium ion only induced color transition of PDA-MP in UV and fluorescence spectra (Supporting Information Figure S8).

Colorimetric Detection of Cadmium Ion. To demonstrate improved sensitivity of PDA-MP compared to previous study, we calculated color response value (CR%) and limit of detection (LOD) using spectroscopic data (Figure 6). The detection limit was estimated at 2.10 μM (3δ/S), where δ represents the standard deviation of the blank measurements, and S is the slope of the intensity versus

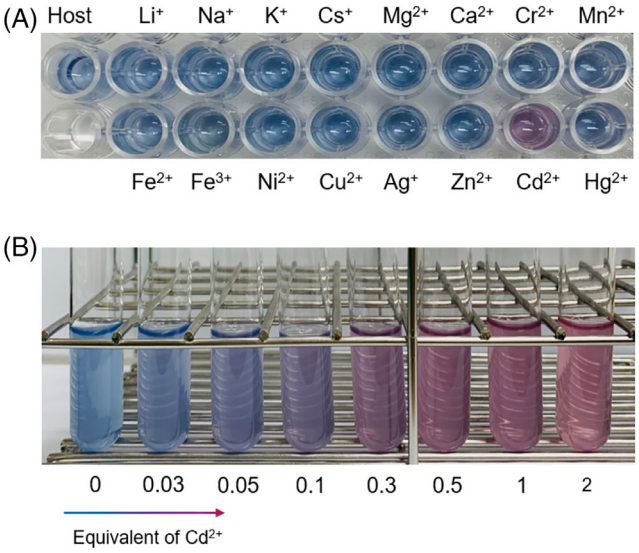

Figure 4. Color transition of PDA-MP (500 μM) (a) in the presence of 1 eq. various metal ions in water; (b) in the presence of various amount of Cd^{2+} (0, 0.03, 0.05, 0.1, 0.3, 0.5, 1 and 2 eq.) in water (200 μM of PDA-MP).

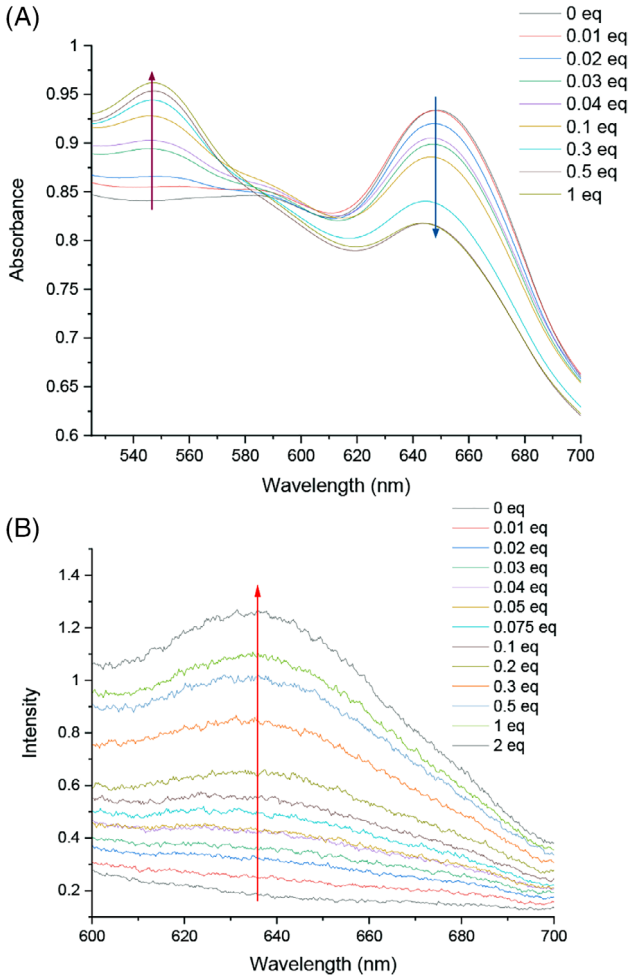

Figure 5. Spectroscopic data (a) UV–vis absorption spectra of PDA-MP (200 μM) in the presence of various equivalents of Cd^{2+} in aqueous solutions; (b) fluorescence spectroscopy titrations of PDA-MP (200 μM) in the presence of various equivalents of Cd^{2+} (excitation at 540 nm, slit: 5 nm/5 nm).

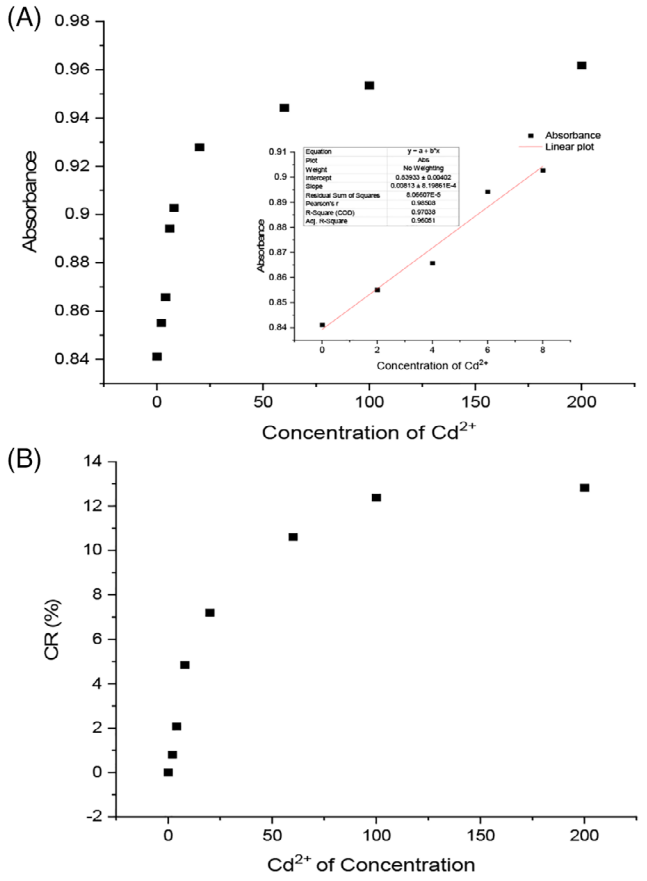

Figure 6. (a) Calibration curve of PDA-MP (200 μM) versus Cd^{2+} concentration. (b) Quantitative colorimetric response of PDA-MP (200 μM) in the presence of various Cd^{2+} concentrations.

sample concentration curve. In a previous study, the LOD was 16.48 μM,[10] whereas the present value (2.10 μM) was dramatically decreased suggesting enhanced sensitivity. Figure 6(b) explains CR%, which shows a linear increase in Cd^{2+} concentration from 2 μM to and the maximal CR value was obtained after adding 60 μM. Additionally Job's plots (continuous variation plots) were carried out (Supporting Information Figure S9), and the molar fractions at 0.65 presented a 2:1 stoichiometry.[14]

Interaction Between PCDA-MP and Cadmium Ion. ^1H NMR titration experiments were performed in $CDCl_3$ (0 eq of Cd^{2+}) and CD_3OD (0.1, 0.2, 0.4, 0.5, 1.0 eq of Cd^{2+}). The addition of Cd^{2+} to PCDA-MP monomer led to a downfield shift of some aromatic proton (H_b, H_c, H_d, H_e, and H_f) peaks and upfield shift of other protons (H_a) (Figure 7 and Supporting Information Table S1). Additionally, Cd^{2+} treatment resulted in broadening of peaks, suggesting that the Cd^{2+} ion interacted with ligand (MP) and not just PCDA moiety.

Conclusion

In the current study, we successfully designed and synthesized a polydiacetylene monomer linked to a simple Cd^{2+} ion chelate. PDA-MP conjugated polymer sensors were prepared via photopolymerization with PCDA-MP and

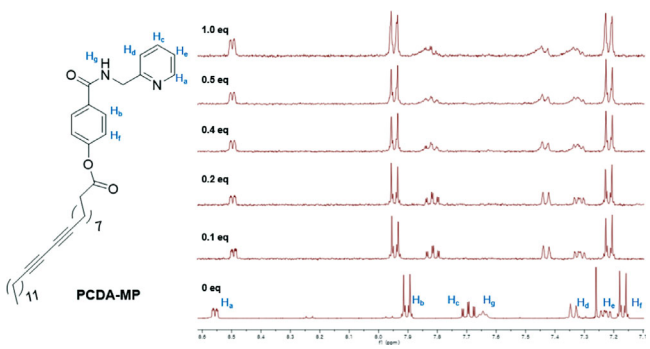

Figure 7. Partial ^1H NMR spectra (400 MHz) of PCDA-MP (2 mM) upon the addition of Cd^{2+} in $CDCl_3$ (0 eq) CD_3OD (0.1, 0.2, 0.4,0.5, 1.0 eq).

PCDA at a ratio of 5:1. This sensor displayed colorimetric and fluorometric changes with Cd^{2+} in aqueous solutions at low detection limits. Moreover, the fluorescence emission increased as the Cd^{2+} concentration increased from 0 to 2 equivalents. This chelate structure can be used in bio-imaging applications of cadmium ion and in the detection of new molecules.

Acknowledgments. This research was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education (No. 2017R1A6A3A04004954) for S. L. and by the Pukyong National University Development Project Research Fund, 2020.

Supporting Information. Additional supporting information may be found online in the Supporting Information section at the end of the article.

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