Metal and Phosphate Ion Recognition using Dipicolylamine-Modified Fluorescent Silica Nanoparticles

Yuji TSUCHIDO, Aya YAMASAWA, Takeshi HASHIMOTO, and Takashi HAYASHITA†

Department of Materials and Life Sciences, Faculty of Science and Technology, Sophia University, 7-1 Kioi-cho, Chiyoda, Tokyo 102-8554, Japan

†To whom correspondence should be addressed.

E-mail: ta-hayas@sophia.ac.jp
Abstract

Dipicolylamine-modified fluorescent silica nanoparticles were prepared by introducing dipicolylamine to the surface of silica nanoparticles possessing terminal amines. We examined the selectivities of dipicolylamine-hydroxycoumarin carbonate (dpa-HCC) and dpa-HCC/fluorescent silica nanoparticles (FSiNP) for metal ions and phosphate anions. The dipicolylamine-modified silica nanoparticles responded to Pb\(^{2+}\) and triphosphate, indicating novel selectivity derived from the assembly effect of dpa-HCC on the silica nanoparticle surface. Surface-modified fluorescent silica nanoparticles are expected to be used as a sensor for environmental and biological applications.

Keywords: silica nanoparticle, dipicolylamine, fluorescent probe, molecular recognition, metal ion, phosphate
Introduction

The development of novel analytical methods for small molecules of environmental or biological interest has witnessed an upsurge over the past decades.\(^1,2\) Many researchers have shown keen interest in the detection of analytical targets, including toxic heavy metal ions or anionic key molecules, such as phosphate derivatives.\(^3,5\) As heavy metal ions pose a threat to human health, it is important to monitor them in water.\(^6,7\) Phosphate anions are one of the most important constituents in living organisms. A variety of sensors for anions, including phosphate ions, have been developed.\(^8-12\) However, most of the sensors require organic solvents because they function via electrostatic interaction or hydrogen bonding for the recognition of analytes. These interactions are weakened drastically in water because of the competing hydration effect. Thus, the detection of such anions as phosphate in water is a challenging task.

Several methods for monitoring these small molecules have been developed. Among them, fluorescent sensors have attracted much attention because of their high sensitivity.\(^13\) We have reported a novel fluorescent molecular recognition sensor composed of phenylboronic acid modified cyclodextrin and phenylboronic acid possessing a pyrene moiety.\(^14\) This supramolecular sensor shows fluorescence intensity changes through the recognition of small molecules. In this study, we developed a fluorescent sensor possessing coumarin and a dipicolylamine moiety. One of the important chemical characteristics of the dipicolylamine moiety is that it forms a chelate complex with metal ions and the dipicolylamine-metal complex forms a bond with phosphates by using the unoccupied coordination sites of the metal complex.
Nanoparticles have also attracted the attention of many researchers because of those attractive characteristics. The advantage of using fluorescent silica nanoparticles is that it is easy to observe the dispersity of nanoparticles by naked eyes because fluorescent dyes can enhance fluorescence signals, thereby increasing sensitivity. And also, it is known that Ru(bpy)$_3^{2+}$ is an excellent doping agent for the synthesis of silica nanoparticles because of its water solubility and positive charge. The Ru(bpy)$_3^{2+}$ doped silica nanoparticles is easy to prepare by centrifugation and other solution treatment processes because of their high density. In addition, their surfaces are easy to modify with various recognition probes. Importantly, one of the advantages of nanoparticles for molecular recognition is the multipoint recognition toward target molecules on the surface of the nanoparticles. We previously reported fluorescent silica nanoparticles (FSiNPs) modified with $\text{M}$ (metal ion)-dpa-HCC for $S. \text{aureus}$ detection. The $\text{M-dpa-HCC/FSiNP}$ complex efficiently formed aggregates with $S. \text{aureus}$. In that study, we observed interesting responses towards metal ions and phosphate anions as well. Here we report differences in selectivity for metal ions and phosphate anions between dpa-HCC and dpa-HCC/FSiNP. We revealed that the novel selectivity was derived from the assembly effect of dpa-HCC on the silica nanoparticle surface.

**Experimental**

**Reagents and chemicals**

2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES), acetonitrile (Luminasol), sodium hydroxide, and sodium nitrate were purchased from Wako Pure Chemical
Industries, Ltd. (Osaka, Japan). 7-Hydroxycoumarin-3-carboxylic acid, 2,2’-dipicolylamine, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM), and formaldehyde (37% aqueous solution) were purchased from Tokyo Chemical Industry, Co., Ltd. (Tokyo, Japan). Acetonitrile, chloroform, and chloroform-\textit{d} were purchased from Kanto Chemical, Co., Inc. (Tokyo, Japan). Sodium dihydrogen phosphate was purchased from Sigma-Aldrich Japan, Co., LLC. (Tokyo, Japan). All other organic solvents and reagents were commercially available with guaranteed grades and used without further purification. Water was doubly distilled and deionized by a Milli-Q water system (WG222, Yamato Scientific Co., Ltd., Tokyo, Japan and Autopure WR-600G, Merck Millipore, MA, USA) before use.

\textbf{Apparatus}

\textsuperscript{1}H NMR spectra were measured with a Lambda GX-500 (JEOL Ltd., Tokyo, Japan) at 300 K. Elemental analysis was performed with a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (PerkinElmer, Inc., MA, USA). All pH values were recorded with a Horiba F-52 pH meter (HORIBA, Ltd., Kyoto, Japan). UV-Vis absorption spectra were measured with a Hitachi U-3900 UV-Vis spectrophotometer (Hitachi High-Technologies, Co., Tokyo, Japan) equipped with a Peltier thermocontroller with a 10-mm quartz cell at 25 °C. Fluorescence spectra were measured with a Hitachi F-7000 fluorescence spectrophotometer (Hitachi High-Technologies, Co., Tokyo, Japan) equipped with a Peltier thermocontroller with a 10-mm quartz cell at 25 °C.

\textit{Synthesis of dpa-HCC}
2,2’-Dipicolylamine (1.025 g, 5.04 mmol) and formaldehyde (37% aqueous solution, 0.441 mL, 5.04 mmol) were dissolved in 30 mL of acetonitrile and refluxed for 2 h. 7-Hydroxycoumarin-3-carboxylic acid (1.072 g, 5.09 mmol) dissolved in 30 mL of acetonitrile was added to the reaction mixture. The reaction mixture was stirred for 2 h and filtered to obtain a highly viscous yellow liquid. The viscous liquid was dissolved in chloroform and extracted with water/chloroform. The organic layer was washed with deionized water and dried *in vacuo* to give a yellow solid (1.31 g, 3.13 mmol, 62.1%). \(^1\)H NMR (Fig. S1, Supporting Information, 500 MHz, CDCl\(_3\)) \(\delta\) (ppm): 8.80 (s, 1H), 8.57 (d, 2H, \(J_{\text{kl}} = 5.7\) Hz), 7.67 (td, 2H, \(J_{\text{hi},\text{ij}} = 7.5, 1.8\) Hz), 7.53 (d, 1H, \(J_{\text{cd}} = 9.0\) Hz), 7.34 (d, 2H, \(J_{\text{hi}} = 7.5\) Hz), 7.21 (t, 2H, \(J_{\text{jk}} = 5.7\) Hz), 7.02 (d, 1H, \(J_{\text{dc}} = 9.0\) Hz), 4.14 (s, 2H), 3.96 (s, 4H); ESI-HRMS Calcd. for \(\text{C}_{23}\text{H}_{18}\text{N}_{3}\text{O}_{5}\) [M-H]: 416.12519, Found: 416.12585. Anal. Calcd. for \(\text{C}_{23}\text{H}_{19}\text{N}_{3}\text{O}_{5}\) (%): C, 66.18; H, 4.59; N, 10.07. Found: C, 66.39; H, 4.60; N, 10.25.

**Scheme 1**

*Synthesis of dpa-HCC-modified fluorescent silica nanoparticles (dpa-HCC/FSiNPs)*

Cyclohexane (22.5 cm\(^3\)), Triton X-100 (5.4 cm\(^3\)), 1-hexanol (5.4 cm\(^3\)), and 20 mM [Ru(bpy)\(_3\)] (1.5 cm\(^3\)) were mixed and sonicated in a homogenizer. The mixture was stirred for 1 h at room temperature. TEOS (0.60 cm\(^3\)) and 30% ammonium hydroxide (1.80 cm\(^3\)) were added and the reaction mixture was stirred for 24 h. The reaction was stopped by adding acetone and the reaction mixture was left to stand to form a suspension. The suspension was centrifuged and washed with acetone, ethanol, and water to obtain the product (FSiNPs).

FSiNPs (60 mg) were dispersed in methanol (10 cm\(^3\)) and APS (240 mg) dissolved in
methanol (10 cm$^3$) was added. Acetic acid (3 cm$^3$) and water (7 cm$^3$) were added to this solution and the entire solution was refluxed for 24 h. The reaction mixture was centrifuged and washed with methanol and water. The precipitate was dried in vacuo to give the product (FSiNP-APS).

FSiNP-APS (106.0 mg) and dpa-HCC (42.7 mg) were dispersed in methanol (30 cm$^3$). DMT-MM (29.7 mg) was added and the reaction mixture was stirred for 16 h at room temperature. The mixture was centrifuged and the precipitate was washed with methanol and water. The precipitate was dried in vacuo to obtain the product (dpa-HCC/FSiNP).

[Fig. 1]

Characterization of nanoparticles

Hydrodynamic diameters of silica nanoparticles were determined by DLS measurements. DLS measurements and ζ-potential measurements were carried out at 25 °C using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, Worcestershire, United Kingdom) at the wavelength of 633 nm and the detection angle of 173° to determine the size of FSiNP, FSiNP-APS and dpa-HCC/FSiNP. The measured autocorrelation function was analyzed by the cumulant method.$^{25}$ The hydrodynamic diameter of silica nanoparticles was calculated by the Stokes-Einstein equation. The standard deviation is given by three independent measurements. The each diameters of FSiNP, FSiNP-APS and dpa-HCC/FSiNP were determined to be 129.0 ± 5.3, 141.3 ± 2.5 and 228.4 ± 5.2 nm (Fig. S2-S4, Supporting Information). The ζ-potential measurements were performed using a capillary ζ-potential cell in the automatic mode. The each ζ-potentials of FSiNP, FSiNP-APS and dpa-HCC/FSiNP were determined to be -8.41 ± 0.35, +1.64 ± 1.14 and +0.28 ± 1.35 mV. The number of amino
group on the surface of FSiNP-APS was determined by the acid-base titration analysis. FSiNP-APS (2.88 mg) was added to 10 mM HCl (1 cm$^3$) and washed well by water. The pH changes of 1.0 mM NaOH (10 cm$^3$) was measured before and after adding FSiNP-APS. The number of amino group on the surface of FSiNP-APS was determined to be $9.2 \times 10^{-7}$ mol/mg. On the assumption that all the amino groups were replaced with dpa-HCC, the number of dpa-HCC in dpa-HCC/FSiNPs was estimated to be $9.2 \times 10^{-7}$ mol/mg.

Results and Discussion

First, to determine the excitation wavelength for fluorescence measurement, the response of dpa-HCC towards zinc ion was measured by UV-Vis spectroscopy (Fig. S5, Supporting Information). The absorbance at 380 nm was increased and a red shift was observed by the addition of metal ions. The isosbestic point was observed at 358 nm. Thus, the excitation wavelength was determined to be 358 nm. The pH profile of dpa-HCC shown in Fig. 2 indicated that the fluorescence change had five states. At low pH (state (a)), the carboxyl moiety and the nitrogen atom of the dipicolylamino moiety were protonated. Fluorescence was weakened because protonated pyridine behaved as an electron acceptor, and photoinduced electron transfer (PET) took place from coumarin to pyridine. When the solution pH was increased, the carboxyl group and the nitrogen atom of pyridine were deprotonated and bound to the hydroxyl group of coumarin (state (b)). When the solution pH was further increased, fluorescence was recovered because the hydroxyl group of coumarin was deprotonated and PET was efficiently inhibited (state (c)). Then, the fluorescence exhibited a maximum because
other pyridine moieties of dipicolylamine were deprotonated and PET was inhibited (state (d)).

When the solution pH was basic, the nitrogen atom of dipicolylamine was deprotonated and the hydrogen bond between dipicolylamine and coumarin disappeared (state (e)). Then, fluorescence intensity was decreased because PET occurred between the lone pair of nitrogen atom and coumarin.

[Fig. 2]

To evaluate selectivity for metal ions, UV-Vis and fluorescence spectra of dpa-HCC with various metal ions were measured (Fig. S6, Supporting Information). The maximum of the fluorescence intensity at 441 nm with various metal ions is shown in Fig. 3. The fluorescence intensity at 441 nm was increased by the addition of Zn$^{2+}$ and Cd$^{2+}$, and decreased by the addition of Pb$^{2+}$, Cu$^{2+}$, Co$^{2+}$, and Ni$^{2+}$. To evaluate the binding affinity of dpa-HCC for metal ion, it was assumed that dpa-HCC forms a 1:1 complex with metal ion. The binding constant of Zn$^{2+}$, Cd$^{2+}$, Ni$^{2+}$, and Cu$^{2+}$ was calculated to be $>10^7$ (ND), $>10^7$ (ND), $>10^7$ (ND), and $1.7 \pm 0.7 \times 10^7$ by the curve fitting method. Fluorescence intensity was increased when 0 eq to 1 eq of metal ion was added, and the intensity was almost constant when more than 1 eq of metal ion was added. These results suggested that dpa-HCC forms a 1:1 complex with metal ions.

[Fig. 3]

Metal ions (Zn$^{2+}$, Cu$^{2+}$) were added to dpa-HCC solution to form Zn-dpa-HCC or Cu-dpa-HCC complex. The response towards phosphate anions (Tri, PPI, Pi) was examined by fluorescence measurement. We evaluated the response towards anions by changing the concentration of the phosphate derivatives (Fig. 4). Fluorescence intensity was decreased when phosphate anion was added to Zn-dpa-HCC solution. On the other hand, the
fluorescence intensity of Cu-dpa-HCC was increased in the presence of PPI and Tri. When Cu²⁺ coordinated to dpa-HCC, the fluorescence intensity of dpa-HCC was decreased because of ligand-to-metal charge transfer (LMCT) from excited coumarin to Cu²⁺. When PPI and Tri were added to this Cu-dpa-HCC complex solution, the binding between dpa-HCC and Cu²⁺ was weakened because of the strong binding of PPI and Tri to the Cu-dpa-HCC complex, resulting in the enhancement of fluorescence intensity. The reason for the difference in fluorescence intensity was that the coordination of PPI or Tri was much stronger than that of Pi.

[Fig. 4]

To discuss the assemble effect of dpa-HCC, we developed dpa-HCC-modified FSiNPs and examined their molecular recognition function. The response of the dpa-HCC/FSiNP complex towards metal ions is depicted in Fig. 5. It is evident that the fluorescence intensities were increased in the presence of Cd²⁺ and Pb²⁺ and decreased in the presence of Cu²⁺, Co²⁺, and Ni²⁺. The response toward metal ions was apparently changed by modifying dpa-HCC on the FSiNP surface and the results showed remarkable selectivity for Pb²⁺. Pb²⁺ was quenched by dpa-HCC, but dpa-HCC/FSiNP showed fluorescence emission toward Pb²⁺. This indicated that the assembly of dpa-HCC on the FSiNP surface enhanced the selectivity for Pb²⁺. In order to investigate the complexation of the Pb²⁺ by dpa-HCC/FSiNP, Pb²⁺ titration measurement was performed (Fig. 6a). The concentration of dipicolylamine at the surface of dpa-HCC/FSiNP could not be calculated. Thus, the real binding constant of dpa-HCC/FSiNP toward Pb²⁺ could not be obtained from this measurement. However, the apparent binding constant, \( K_{\text{app,bind}} \), which was calculated by the following formula provided by Damos et al. and Endo et al., assuming a Langmuir isotherm.\(^{26,27} \)
\[
\frac{[\text{Pb}^{2+}]}{\Delta F} = \frac{1}{(K_{\text{app,bind}} \times \Delta F_{\text{max}})} + \frac{[\text{Pb}^{2+}]}{\Delta F_{\text{max}}}
\]

where \([\text{Pb}^{2+}]\) is the total concentration of \(\text{Pb}^{2+}\), \(\Delta F\) is the difference of fluorescence in the presence and absence of the \(\text{Pb}^{2+}\) \((= F - F_0)\), and \(\Delta F_{\text{max}}\) is the maximum value of \(\Delta F\). The plot of \([\text{Pb}^{2+}]/(\Delta F/F_0)\) versus \([\text{Pb}^{2+}]\) for \(\text{dpa-HCC/FSiNP}\) was shown in Fig. 6b. From using the slope and intercept of the Langmuir plot, the value of \(K_{\text{app,bind}}\) was estimated to be \(8.6 \times 10^3\) L mol\(^{-1}\).

Although the detailed mechanism remains unclear, the multipoint interaction of dipicolylamine moieties with \(\text{Pb}^{2+}\) seems to induce the novel selectivity for \(\text{Pb}^{2+}\).

[Fig. 5]

[Fig. 6]

We also evaluated the response of the \(\text{M-dpa-HCC/FSiNP}\) complex towards various anions (Pi, PPI, and Tri) (Fig. 7). The \(\text{dpa-HCC/FSiNP}\) complexes with \(\text{Zn}^{2+}\) or \(\text{Cu}^{2+}\) were examined at pH 7.3. The fluorescence spectra are shown in Figure S7. When anions were added to \(\text{Zn-dpa-HCC/FSiNP}\) solution, the little changes of fluorescence intensities of \(\text{Zn-dpa-HCC/FSiNP}\) were observed. On the other hand, for \(\text{Cu-dpa-HCC/FSiNP}\) solution, the fluorescence intensities of \(\text{Cu-dpa-HCC/FSiNP}\) were increased in the presence of PPI and Tri. This indicated that the coordination of \(\text{Cu}^{2+}\) quenched the fluorescence due to LMCT. Because of the coordination of phosphates with \(\text{Cu}^{2+}\), the binding of \(\text{dpa-HCC}\) and \(\text{Cu}^{2+}\) was weakened and the fluorescence was selectively recovered. In order to discuss phosphates selectivity, phosphate titration experiments were carried out (Fig. 8a). Fluorescence changes by PPI and Tri addition were almost same and saturated about 0.2 mM. On the other hand, fluorescence change of Pi was quite a little. The plot of \([\text{anion}]/(\Delta F/F_0)\) versus \([\text{anion}]\) for \(\text{Cu-dpa-HCC/FSiNP}\) was shown in Fig. 8b. The apparent binding constants, \(K_{\text{app,bind}}\) between
Cu-dpa-HCC/FSiNP and Pi, PPI and Tri were estimated to be $1.1 \times 10^3$, $2.6 \times 10^3$ and $2.7 \times 10^3$ L mol$^{-1}$. These results showed that $K_{\text{app\_bind}}$ of PPI and Tri was higher than that of Pi. PPI and Tri possess two or more phosphate units in each molecule, however, Pi has only one phosphate unit in the molecule. This result indicated that the multipoint recognition of PPI and Tri by Cu-dpa-HCC/FSiNP accelerated the selectivity for phosphates in water.

Conclusions

We have developed dipicolylamine-modified fluorescent silica nanoparticles. The dpa-HCC/FSiNPs showed strong fluorescence upon recognition of Pb$^{2+}$ whereas dpa-HCC alone did not recognize Pb$^{2+}$. Although the reason for the enhanced selectivity for Pb$^{2+}$ was not clear, dpa-HCC assembled on the FSiNP surface affected the recognition behavior for metal ions. In addition, Cu-dpa-HCC/FSiNPs were found to selectively recognize Tri at pH 7.3. These selective recognitions were apparently induced by the multipoint recognition function of dpa-HCC assembled on the FSiNP surface.

Acknowledgements

This work was financially supported by Grants-in-Aid for Scientific Research (C) (Grant No. 15K05548) and Early-Career Scientists (Grant No. 18K14255) from Japan Society for the
Promotion of Science (JSPS) and a Grant-in Aid for Scientific Research (A) (Grant No. 26248038) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

**Supporting Information**

$^1$H NMR, DLS, UV-Vis, and fluorescence spectra are available in Supporting Information. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.
References


**Figure Captions**

Fig. 1  Schematic representation of dpa-HCC/FSiNP.

Fig. 2  pH profile of dpa-HCC in 1% AN-99% water (v/v) ($\lambda_{ex}$=358 nm). $F-F_0$ is fluorescent intensity difference at 441 nm. [dpa-HCC] = 0.01 mM, [NaNO$_3$] = 100 mM, [HEPES] = 5 mM, pH 7.3, 25°C.

Fig. 3  Selectivity of dpa-HCC toward metal ions in 1% AN-99% water (v/v) ($\lambda_{ex}$=358 nm). $F-F_0$ is fluorescent intensity difference at 441 nm. [dpa-HCC] = 0.01 mM, [(metal)(NO$_3$)$_n$] or [K$_2$PtCl$_4$] = 0.01 mM, [NaNO$_3$] = 100 mM, [HEPES] = 5 mM, pH 7.3, 25°C.

Fig. 4  Selectivity of M-dpa-HCC for phosphate anions in 1% AN-99% water (v/v) ($\lambda_{ex}$ = 353 nm). $F - F_0$ is fluorescent intensity difference at 441 nm. [dpa-HCC] = 0.01 mM, [M(NO$_3$)$_2$] = 0.02 mM, (M = (a) Zn$^{2+}$, (b) Cu$^{2+}$) [anion] = 1.0 mM, [HEPES] = 5 mM, pH 7.3, 25°C.

Fig. 5  Selectivity of dpa-HCC/FSiNP complex for metal ions in water ($\lambda_{ex}$ = 380 nm). $F-F_0$ is fluorescent intensity difference at 447 nm. [dpa-HCC/FSiNP] = 0.1 mg/mL, [(metal)(NO$_3$)$_n$] or [K$_2$PtCl$_4$] = 5.0 mM, [NaNO$_3$] = 100 mM, [HEPES] = 5 mM, pH 7.3, 25°C.

Fig. 6 (a) Titration of Pb$^{2+}$ toward dpa-HCC/FSiNP in water (v/v) ($\lambda_{ex}$ = 380 nm). $F - F_0$ is fluorescent intensity difference at 450 nm. (b) A plot of [Pb$^{2+}$]/($\Delta F/F_0$) versus [Pb$^{2+}$]. [dpa-HCC/FSiNP] = 0.1 g/mL, [HEPES] = 5 mM, pH 7.3, 25°C.

Fig. 7  Selectivity of M-dpa-HCC/FSiNP for phosphate anions in water (v/v) ($\lambda_{ex}$ = 380 nm).
$F - F_0$ is fluorescent intensity difference at 450 nm. $[\text{dpa-HCC/FSiNP}] = 0.1$ g/mL, $[\text{M(NO}_3)_2] = 0.02$ mM, (M = (a) Zn$^{2+}$, (b) Cu$^{2+}$) [anion] = 1.0 mM, [HEPES] = 5 mM, pH 7.3, 25°C.

Fig. 8 (a) Titration of phosphate anions toward Cu-dpa-HCC/FSiNP in water (v/v) ($\lambda_{ex} = 380$ nm). $F - F_0$ is fluorescent intensity difference at 450 nm. (b) A plot of [anion]/($\Delta F/F_0$) versus [anion]. $[\text{Cu-dpa-HCC/FSiNP}] = 0.1$ g/mL, $[\text{Cu(NO}_3)_2] = 0.02$ mM, [HEPES] = 5 mM, pH 7.3, 25°C.

Scheme 1 Synthesis of dpa-HCC.
Fig. 1 Schematic representation of dpa-HCC/FSiNP.
Fig. 2 pH profile of dpa-HCC in 1% AN-99% water (v/v) ($\lambda_{ex}$=358 nm). $F-F_0$ is fluorescent intensity difference at 441 nm. [dpa-HCC] = 0.01 mM, [NaNO$_3$] = 100 mM, [HEPES] = 5 mM, pH 7.3, 25°C.
Fig. 3 Selectivity of dpa-HCC toward metal ions in 1% AN-99% water (v/v) ($\lambda_{ex}$=358 nm).

$F - F_0$ is fluorescent intensity difference at 441 nm. [dpa-HCC] = 0.01 mM, [(metal)(NO$_3$)$_3$] or [K$_2$PtCl$_4$] = 0.01 mM, [NaNO$_3$] = 100 mM, [HEPES] = 5 mM, pH 7.3, 25°C.
Fig. 4 Selectivity of M-dpa-HCC for phosphate anions in 1% AN-99% water (v/v) ($\lambda_{ex} = 353$ nm). $F - F_0$ is fluorescent intensity difference at 441 nm. [dpa-HCC] = 0.01 mM, [M(NO$_3$)$_2$] = 0.02 mM, (M = (a) Zn$^{2+}$, (b) Cu$^{2+}$) [anion] = 1.0 mM, [HEPES] = 5 mM, pH 7.3, 25°C.
Fig. 5 Selectivity of dpa-HCC/FSiNP complex for metal ions in water ($\lambda_{ex} = 380$ nm). $F-F_0$ is fluorescent intensity difference at 447 nm. $[\text{dpa-HCC/FSiNP}] = 0.1$ mg/mL, $[(\text{metal})(\text{NO}_3)_n]$ or $[\text{K}_2\text{PtCl}_4] = 5.0$ mM, $[\text{NaNO}_3] = 100$ mM, [HEPES] = 5 mM, pH 7.3, 25°C.
Fig. 6 (a) Titration of Pb$^{2+}$ toward dpa-HCC/FSiNP in water (v/v) ($\lambda_{ex} = 380$ nm). $F - F_0$ is fluorescent intensity difference at 450 nm. (b) A plot of [Pb$^{2+}$]/($\Delta F/F_0$) versus [Pb$^{2+}$]. $[\text{dpa-HCC/FSiNP}] = 0.1$ g/mL, [HEPES] = 5 mM, pH 7.3, 25°C.
Fig. 7 Selectivity of M-dpa-HCC/FSiNP for phosphate anions in water (v/v) ($\lambda_{ex} = 380$ nm). $F - F_0$ is fluorescent intensity difference at 450 nm. [dpa-HCC/FSiNP] = 0.1 g/mL, [M(NO$_3$)$_2$] = 0.02 mM, (M = (a) Zn$^{2+}$, (b) Cu$^{2+}$) [anion] = 1.0 mM, [HEPES] = 5 mM, pH 7.3, 25°C.
Fig. 8 (a) Titration of phosphate anions toward Cu-dpa-HCC/FSiNP in water (v/v) ($\lambda_{\text{ex}} = 380$ nm). $F - F_0$ is fluorescent intensity difference at 450 nm. (b) A plot of [anion]/($\Delta F/F_0$) versus [anion]. [Cu-dpa-HCC/FSiNP] = 0.1 g/mL, [Cu(NO$_3$)$_2$] = 0.02 mM, [HEPES] = 5 mM, pH 7.3, 25°C.
Scheme 1 Synthesis of dpa-HCC.
Graphical Index