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ORIGINAL PAPER

Synthesis of Pyridine-Based 1,3,4-Oxadiazole Derivative as Fluorescence Turn-On Sensor for High Selectivity of Ag^+

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Abstract An oxadiazole derivative(OXD) containing symmetrical pyridine-2-formamidophenyl-binded moiety was synthesised as fluorescence turn-on sensor **OA1**. Its ultraviolet–visible(UV–vis) and fluorescent spectra(FS) gave prominent fluorescence enhancement only for monovalent silver ion(Ag^+) in HEPES buffer solution (10 mM, pH=7.0, DMF-H_2O, 9:1, v/v), which indicated the photo-induced electron transfer(PET) occurred from the donor of pyridine-2-formamidophenyl group to oxadiazole fluorophore. The present study demonstrated that **OA1** was a viable candidate as fluorescent receptor for a new Ag^+ sensor. And the results of fluorescent spectral titration showed this sensor formed 1:1 complex with Ag^+.

Keywords Fluorescent sensor · Silver ion · Oxadiazole · Photo-induced electron transfer

Introduction

Selective sensors for detection and quantification of various metal ions and organic structures have received considerable attention in many fields such as environmental and security monitoring, waste management, nutrition, and clinical toxicology [1–6]. An ideal sensory system should contain multiple members that respond to analytes with high sensitivity and selectivity. Although previous work has achieved a wide variety of chemical [7–12] and physical [13–17] sensors for the detection of cations, improving the analysis selectivity out of interference from coexisting metal ions has been challenging. Therefore, the rational design and synthesis of efficient sensors to selectively recognize targets is the hot topic in molecular recognition studies. Because of high sensitivity, fluorescent reagents have been used satisfactorily for the fluorometric detection of most transition or heavy metal ions [18–27], and fluorescent spectroscopy is becoming more and more important for chemical trace detection [20, 28–30].

Among different fluorescent cation sensing probes, photoinduced electron transfer(PET)-type receptors have been proved highly successful [2, 31–36], which are devised to covalently link fluorophores by means of non-conjugating spacer groups and reversibly switch fluorescent intensity by binding cations. Usually, highly selective probes for transition or heavy elements that give a positive response rather than fluorescent quenching upon analyst binding are preferred to promote the sensitivity factor. Consequently, the design of such turn-on silver ion(Ag^+) sensors is an intriguing challenge since many transition elements often causes fluorescent quenching [37].

In view of above requirement and as part of an ongoing investigation focused on the preparation and utilization of small-molecule electroluminescent organisms, we reported an oxadiazole derivative(OXD) as Ag^+ fluorescent sensor **OA1** herein, which gave a positive response to Ag^+ in HEPES buffer solution(10 mM, pH=7.0, DMF-H_2O, 9:1, v/v). In the design of **OA1**, we employed 1,3,4-oxadiazole as fluorophore because of its excellent photophysical properties and chemical stabilities [38]. Two pyridine-2-formamidophenyl were symmetrically bound on C-2 and C-5 of 1,3,4-oxadiazole, in which the nitrogen atom of pyridine ring was both the cation receptor and the quencher via PET. Acceptance of the metal ion sequestered the lone pairs in nitrogen atom and rebuilt rigid molecular system, which stopped the PET quenching and produced a

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fluorescent enhancement in the 1,3,4-oxadiazole emission. Sensor **OA1** shown in Scheme 1 provided high sensitivity and large optics signaling changes for Ag^+.

Experimental

Reagents

All reagents were of analytical grade and used without purification.

Characterization Methods

Thin layer chromatography(TLC) plates were visualized with UV light to monitor the completion of all preparation reactions. Whatman silica gel-60 plates of 1 mm thickness were used as the solid phase for TLC. Melting points(M. p.) of prepared compounds were measured on an X4 Micro-melting point apparatus. A Bruker DRX500 spectrometer recorded ^{1}H NMR spectra of objective products at 300 MHz. ^{1}H chemical shifts were reported in ppm down-field from tetramethylsiane(TMS, δ scale with the solvent resonances as internal standards). An HP 1100LC-MSD high performance system was utilized to obtain atmospheric pressure ionization mass spectra(API-MS) of target compounds.

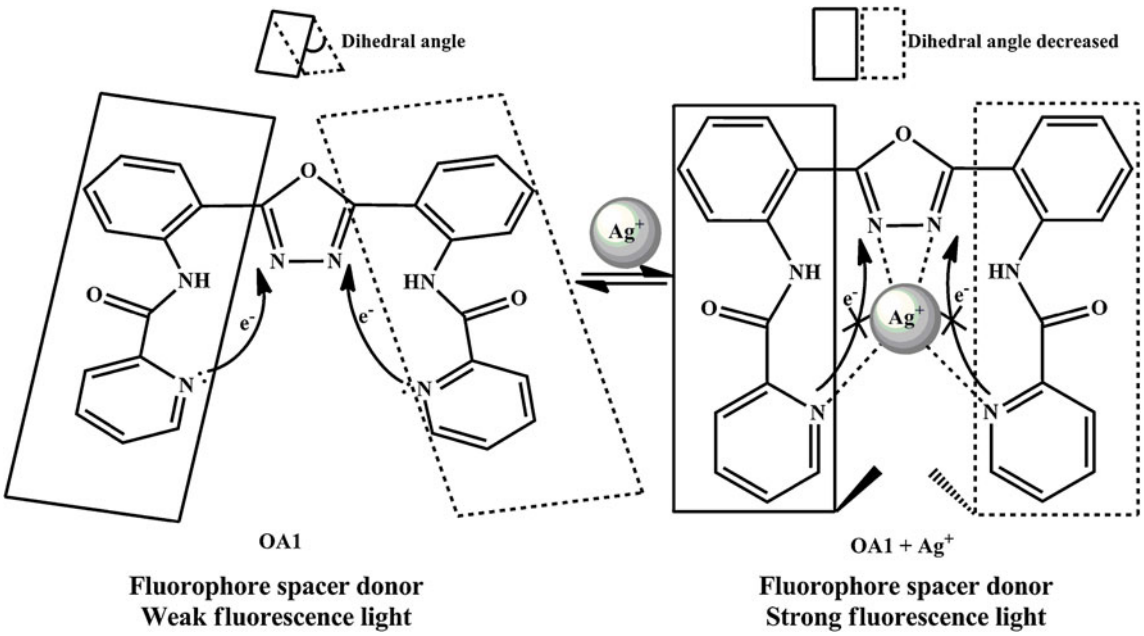
Synthesis

Synthetic procedures of **OA1** were shown in Scheme 2.

o-aminobenzoic Acid (1) *o*-nitrobenzoic acid (33.4 g, 0.2 mol) and distilled water (100 mL) were put into a 250 mL four-neck flask, stirred with sodium hydroxide solution (15 %) dropping until the precipitates were dissolved completely, and heated to 50 °C. Pd/C (3 %) was used as catalyst, and hydrazine hydrate (24 mL, 0.4 mol) was added to above reaction mixture dropwise. Then the reaction temperature was increased to 60 °C for 6 h. Cooled filtrate was adjusted to pH 3~4 with hydrochloric acid (10 %). Finally, the filtered solid was recrystallized from ethanol to obtain light yellow crystal (21 g, 75 %). M. p., 195~196 °C; Found, 193~195 °C [39]. ^{1}H NMR (300 MHz, DMSO-d6, TMS) δ: 8.57 (s, 2H), 7.68 (d, J=8.04 Hz, 1H), 7.21 (t, J=8.4 Hz, 1H), 6.73 (d, J=8.4 Hz, 1H), 6.49 (t, J=8.07 Hz, 1H).

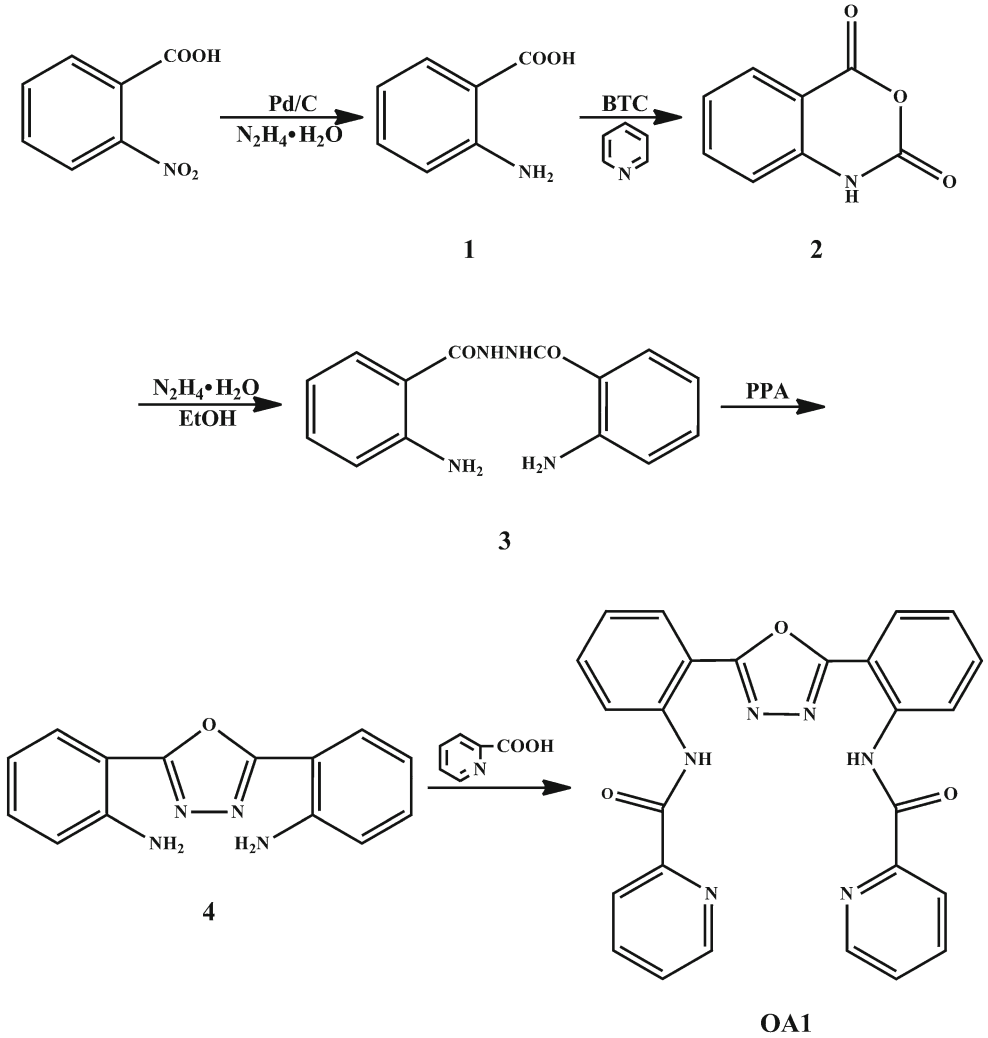
Isatoic Anhydride (2) *o*-aminobenzoic acid (21 g, 0.15 mol) and acetonitrile (100 mL) were added into a 250 mL four-neck flask, and heated to 50 °C. Meanwhile, methylene chloride solution containing pyridine (25 mL, 0.3 mol) and triphosgene (15.16 g, 0.05 mol) was dropped. Then the reaction was kept 50~55 °C for 2 h. Finally, the solvent was removed under reduced pressure and the residual solid was recrystallized from ethanol to yield white product (12.3 g, 50 %). M. p., 218 °C; Found, 220~230 °C [40]. ^{1}H NMR (300 MHz, DMSO-d6, TMS) δ: 11.71 (s, 1H), 7.92 (d, J=7.86 Hz, 1H), 7.74 (t, J=7.78 Hz, 1H), 7.25 (t, J= 7.5 Hz, 1H), 7.15 (d, J=8.22 Hz,1H).

N,N'-bis(2-aminobenzoyl)hydrazide (3) Isatoic anhydride (11.2 g, 68.7 mmol) and absolute alcohol (60 mL) were



Scheme 1 Proposed complex formation between Ag^+ and **OA1**

Scheme 2 Synthetic procedures of **OA1**



put into a 100 mL four-neck flask, and hydrazine hydrate (2 mL, 34.3 mmol) was added dropwise at room temperature. The refluxing reaction lasted 4 h until TLC indicated the complete transformation, then was cooled down. Recrystallization for filtered solid from DMF/H_2O would afford the target as brown crystal (6.7 g, 72.2 %). M. p., 208 ~211 °C; Found, 220~230 °C [41]. ^{1}H NMR (300 MHz, DMSO-d6, TMS) δ: 10.02 (s, 1H), 7.60 (d, J=7.86 Hz, 2H), 7.18 (t, J=7.11 Hz, 2H), 6.73 (d, J=8.25 Hz, 2H), 6.53 (t, J= 7.14 Hz, 2H), 6.41 (s, 4H).

2,5-bis(2-aminophenyl)-1,3,4-oxadiazole (4) In a 100 mL four-neck flask, *N*,*N*'-*bis*(2-aminobenzoyl)-hydrazide (4.1 g, 15.2 mmol) and polyphosphoric acid (PPA, 10 mL) were heated quickly to 160 °C and reacted for 2 h. Following, system temperature was decreased to 50 °C. Ice water (100 mL) was added slowly after completed reaction was detected by TLC. The pH was adjusted to 5 with sodium hydroxide solution (15 %). The filtered solid was recrystallized from DMF/H_2O to bring brown needlelike crystal (3.2 g, 83.6 %). M. p., 227~230 °C; Found, 228~230 °C [41]. ^{1}H NMR (300 MHz, DMSO-d6, TMS) δ: 7.87 (d, J=7.89 Hz, 2H), 7.28 (t, J=7.14 Hz, 2H), 6.92 (d, J=8.4 Hz, 2H), 6.76 (s, 4H), 6.71 (t, J=8.04 Hz, 2H). API-MS $[M + H]^+$ *m/z*: 253.2.

2,5-bis(pyridine-2-formamidophenyl)-1,3,4-oxadiazole (OA1) 2,5-*bis*(2-aminophenyl)-1,3,4-oxadiazole (0.85 g, 3.37 mmol), pyridine-2-formic acid (1.28 g, 10.11 mmol), triphenyl phosphite (TPPi, 1.9 mL, 6.74 mmol) and pyridine (10 mL) reacted in a 100 mL four-neck flask at 50 °C for 16 h. Additional TPPi (0.9 mL, 3.37 mmol) joined in the reaction and refluxing continued for another 3 h. The solvents were evaporated and benzene (5 mL) was utilized to wash the residual. Recrystallization for filtered solid from chloroform/ether would produce light yellow crystal (0.96 g, 61.7 %). M. p., 244~246 °C; Found, 249~251 °C [41]. ^{1}H NMR (300 MHz, DMSO-d6, TMS) δ: 12.64 (s, 2H), 8.87 (t, J=4.5 Hz, 4H), 8.28 (m, 4H), 8.14 (t, J=7.8 Hz, 2H), 7.76 (m, 4H), 7.40 (t, J=7.5 Hz, 2H). API-MS $[M + H]^+$ *m/z*: 463.3.

General Spectroscopic Procedures

N-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid(HEPES) from Calbiochem and $AgNO_3$ (99.998 %) were purchased and used as received. Millipore filtered water was used to prepare all aqueous solutions. All spectroscopic measurements were performed at neutral pH with 10 mM HEPES buffer solution adjusting. An Orion glass electrode, calibrated prior to use, was employed to record solution pH. Silver solutions were prepared from 10 mM stock solutions of $AgNO_3$. Stock solution of **OA1** (10 mM in different solvent such as DMF, cyclohexane, toluene, THF, ethyl acetate, acetone, acetonitrile, pyridine, ethylene glycol, monomethyl ether) was prepared. After addition of this stock solution to aqueous buffers, the resulting solution contained 0.1 % solvent for fluorescence and 1 % solvent for absorption measurements. The KaleidaGraph software package was used to manipulate all spectral data.

Optical Absorption Spectroscopy

UV-visible spectra were collected on an Agilent 8453 diode array spectrophotometer which was controlled by a Pentium PC running manufacturer supplied software package. A circulating water bath was used during acquisition to maintain the temperature at 25.0±1.0 °C. Samples were contained in 1 cm path length quartz cuvettes (3.5 mL volume). All manipulations were performed at least three times.

Fluorescence Spectroscopy

Fluorescence spectra(FS) were obtained with a Perkin Elmer LS-55 fluorescence spectrophotometer linked to a Pentium PC running SpectraCalc software package. A rhodamine quantum counter and manufacturer supplied photomultiplier curves were used to normalize the excitation and emission spectra, respectively. A circulating water bath was conducted during all experiments to regulate the temperature at 25.0± 1.0 °C. Spectra were acquired with 3 nm slit widths and a 600 nm/min scan speed. All measurements were carried out at least in triplicate.

Selective Fluorescence Sensing of Molecular Sensor OA1 for Ag^+

In order to ascertain the affect of metal ions on the fluorescence of molecular sensor, a solution of 5.0 μM **OA1** in the buffer containing cation of interest was prepared and the emission spectrum was recorded. The selectivity of **OA1** for Ag^+ against a background of various alkali, alkaline earth, transition metal ions, and Al^{3+} or Pb^{2+} was also investigated by using FS. Aqueous metal ion solutions of Al^{3+}, Ba^{2+}, Cd^{2+},, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+ and Zn^{2+} were prepared from their chloride salts. The Pb^{2+} was prepared from lead nitrate. A solution of Cr^{3+} was prepared from chromium acetate and stored at pH1. Solution of Fe^{2+} was prepared immediately before use with ferrous ammonium sulfate and water that was thoroughly purged with Ar. All stock solutions were approximate 10 mM. In a typical experiment, the emission spectrum of free **OA1** was recorded. A 20 μL aliquot of a 10 mM metal solution was then added to a 5.0 μM solution of **OA1** (3 mL) and the emission spectrum was recorded with excitation at 370 nm. Subsequently, a 20 μL portion of 10 mM $AgNO_3$ was added and the emission spectrum was obtained. For all experiments, the spectra were normalized with respect to free **OA1** and manipulated to each condition at least for three times.

Ag^+ Binding Studies by Fluorescence Spectroscopy

Metal-binding titration experiments were done and the method of continuous variations(Job's analyses) [42] was applied to calculate the correlative stoichiometry of **OA1**-Ag^+ complex according to the following equation.

$$\log\frac{F-F_{\min}}{F_{\max}-F} = n\log[Ag^+] + B$$

In this equation, $F_{\min}$, $F_{\max}$ and F referred to the fluorescent intensity of metal-free **OA1**, **OA1** with excessive Ag^+ and **OA1** with Ag^+ at any concentration between the former two, respectively. n meant the stoichiometry of **OA1**-Ag^+ complex. In a typical titration, 3 μL aliquots of a 1 mM $AgNO_3$ solution in water were added to a 5.0 μM **OA1** solution in DMF and the fluorescence changes at 368 nm were plotted against equivalents of Ag^+ added. All processes were operated at least with three replicates to obtain statistical analysis.

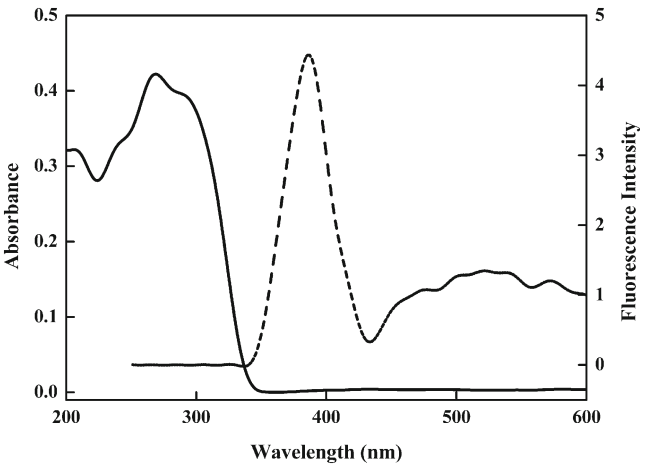


Fig. 1 Absorption and fluorescence spectra of **OA1** in HEPES buffer solution (10 mM, pH=7.0, DMF-H_2O, 9:1, v/v)

Table 1 Excitation and emission spectral data of **OA1** in different solvents at room temperature

Solvent	Absorption maximum(nm)	Fluorescence maximum(nm) (λ_{max}=370 nm)	Stokes shift(nm)
Cyclohexane	269	368	99
Toluene	281	368.5	87.5
THF	288	371.5	83.5
Ethyl acetate	290	371.5	81.5
Acetone	293	375	82
Acetonitrile	290	377.5	87.5
Pyridine	297	378	81
Ethylene glycol	299	378.5	79.5
Monomethyl ether	302	383	81

Results and Discussion

Spectroscopic Properties of Molecular Sensor OA1

The maximum excitation and emission wavelengths of sensor **OA1** in HEPES buffer solution (10 mM, pH=7.0, DMF-H_2O, 9:1, v/v) at room temperature were at 269 nm and 372 nm respectively (Fig. 1).

Meanwhile, the excitation and emission spectra of above **OA1** system have been studied in various solvents with different polarity and the spectral data have been collected (Table 1). As was typical of a charge-transfer transition, an increase in the polarity of medium led to a Stokes shift of excitation maximum. While the change of solvent from cyclohexane to monomethyl ether resulted in a shift of excitation maximum by around 33 nm, and the magnitude of spectral shift was 15 nm in fluorescence. The effect of medium polarity on absorption maximum was more pronounced than that on fluorescence maximum. This meant the ground state of **OA1** should have more significant polarity than that of the emitting one.

Fluorescence Sensing of Molecular Sensor OA1 for Ag^+

The fluorescence sensing abilities were primarily investigated by adding different cations (Ag^+, Al^{3+}, Ba^{2+}, Cd^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Hg^{2+}, K^+, Mg^{2+}, Mn^{2+}, Na^+, Pb^{2+} and Zn^{2+}) to the HEPES buffer solution (10 mM, pH=7.0, DMF-H_2O, 9:1, v/v) containing molecular sensor **OA1**. When 10 equivalent(equiv.) free Ag^+ was added into the buffer solution including **OA1** (5.0 μM), the sensor responded with dramatic fluorescent enhancement contrast to the slight fluorescent change even quenching caused by the other cations (Fig. 2). Meanwhile, the addition of metal ions did not shift the emitting wavelength of **OA1** and all the fluorescent responses were transient without time dependence. Therefore, fluorescent sensor **OA1** exhibited high selectivity and sensitivity for Ag^+.

The fluorescent decrease of **OA1** caused by selected cations except Ag^+, Cd^{2+}, Pb^{2+} and Mn^{2+} was consistent with the behavior of numerous turn-off molecular sensors. PET theory could explain this phenomenon. The nitrogen atom of pyridine ring in **OA1** was the cation receptor which would provide lone pairs and the electron transfer was switched off during binding process between **OA1** and cation. To Ag^+, Cd^{2+}, Pb^{2+} and Mn^{2+}, the rebuilding of rigid molecular system for sensor-metal complex might more

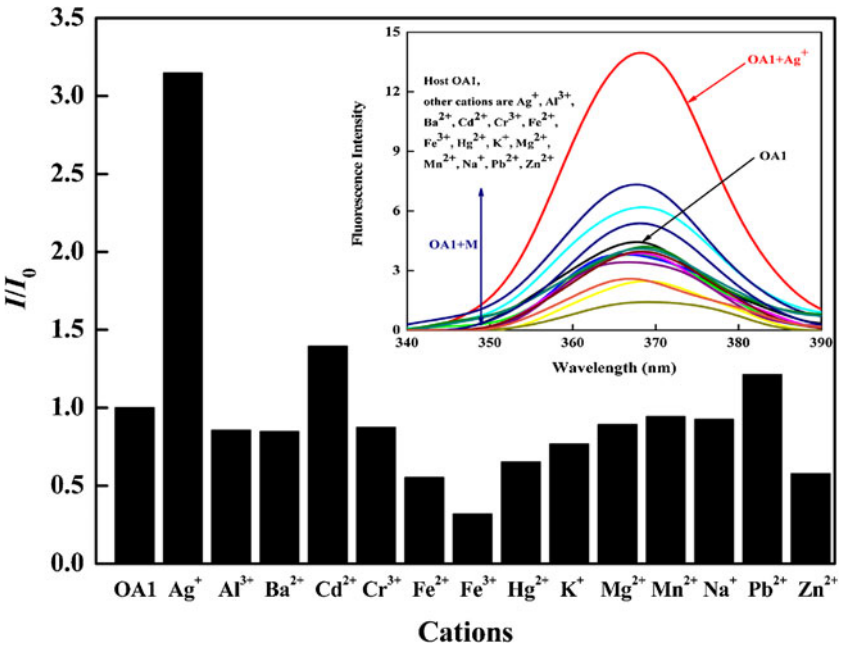


Fig. 2 Relative fluorescence intensity changes of **OA1** (5.0 μM) in the presence of excessive selected cations (10equiv.) in HEPES buffer solution (10 mM, pH=7.0, DMF-H_2O, 9:1, v/v) at room temperature. Inset: Fluorescence spectra of **OA1** (5.0 μM) in the presence of excessive selected cations (10equiv.) in HEPES buffer solution (10 mM, pH=7.0, DMF-H_2O, 9:1, v/v) at room temperature. The excitation was at 370 nm

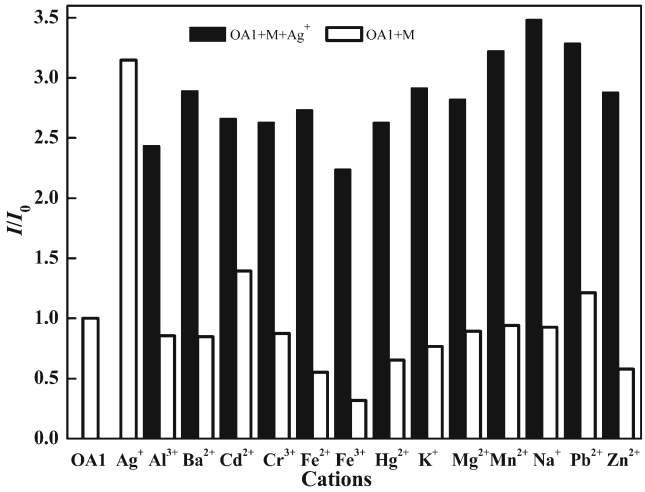


Fig. 3 Relative fluorescence intensity changes of **OA1** (5.0 μM) to Ag^+ (10equiv.) in the presence of selected cations (10equiv.) in HEPES buffer solution (10 mM, pH=7.0, DMF-H_2O, 9:1, v/v) at room temperature. The excitation was at 370 nm

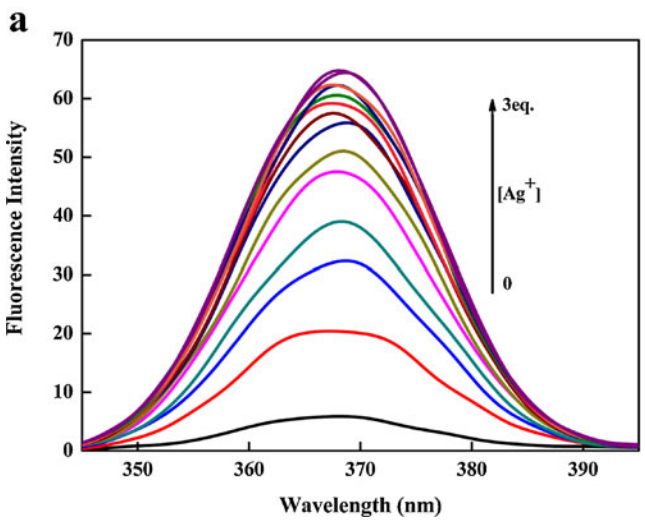
powerful than PET, and a fluorescent increase in the 1,3,4-oxadiazole emission occurred.

An important feature of sensor was its high selectivity toward analyte over other competitive species. In order to investigate the silver recognition abilities of **OA1**, we carried out interference experiments in the same HEPES buffer solution to discuss the competition effects from above selected metal ions. As has been argued, all other cations excluded Ag^+ slightly enhanced even weakened the fluorescence intensity of HEPES buffer solution containing 5.0 μM **OA1**. But when Ag^+ followed to be added into the mixed solution, the fluorescence intensity increased greatly and immediately which was comparable to that of sensor solution with Ag^+ fed into directly. These results indicated that the selectivity of **OA1** for Ag^+ was hardly affected by the coexisted metal ions as shown in Fig. 3.

As **OA1** shown specific selectivity for Ag^+, a series of experiments were planned to discuss the Ag^+ recognition capability and mechanism of **OA1**. The binding properties of sensor **OA1** with Ag^+ were studied by fluorescence spectral titration experiments (Fig. 4a) and the method of continuous variations(Job's analyses) was further used to gain an insight into the stoichiometry of **OA1**-Ag^+ complex (Fig. 4b).

Figure 4a showed the typical fluorescence response spectra of sensor **OA1** in HEPES buffer solution (10 mM, pH= 7.0, DMF-H_2O, 9:1, v/v) with increasing Ag^+ concentrations and the background signal of HEPES alone was deducted to evaluate the fluorescent response of proposed assay. The sensing system exhibited a significant change in fluorescence in the presence of different concentrations of Ag^+. The bottom curve was measured in the absence of Ag^+, where the sensing system had a very weak emission. When differernt concentrations of Ag^+ were added into the solution of **OA1**, a drastic increase in the fluorescence emission was observed.

The intensity of excimer emission increased correspondingly with an increase in Ag^+ concentrations as revealed in Fig. 4b, indicating the formation of **OA1**-Ag^+ complex which stopped the transferring of lone pairs and rebuilt the rigid molecular system as were illustrated in Scheme 1. Once the concentration of Ag^+ was over 5.0 μM, the fluorescence intensity reached a plateau which suggesting the saturated recognition sites of Ag^+ binding. According to



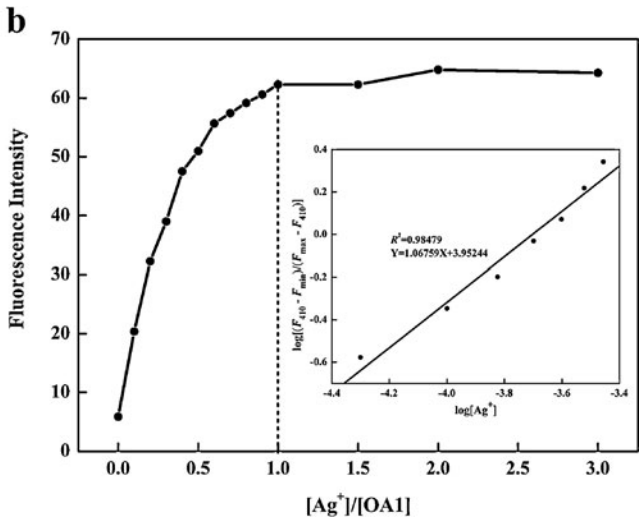


Fig. 4 **a** Fluorescence enhancement of **OA1** (5.0 μM) with different concentrations of Ag^+ (3equiv.) added in HEPES buffer solution (10 mM, pH=7.0, DMF-H_2O, 9:1, v/v) at room temperature. **b** A Job plot of **OA1** and Ag^+ at 368 nm which indicated that the stoichiometry of **OA1**-Ag^+ complex was 1:1. Inset: Linear fitting curve of the changes in fluorescence intensity at 368 nm of **OA1** versus increasing Ag^+ concentration. The excitation was at 370 nm

Job's analyses, the linear fitting curve in the inset further elucidated the binding mode between sensor **OA1** and Ag^+ that a stable 1:1 **OA1**-Ag^+ complex was obtained.

It was clear that **OA1** could be a potential sensor candidate for Ag^+. With the industrialization continuing, silver was applied to mirror, jewelry, medicine and military affairs etc. widely so that it would be released to the environment in various forms especially ionic and masked threat to people's health came into being by the combination of Ag^+ with some enzymes or DNA in organism. **OA1** devised simply provided us a highly selective and sensitive fluorescence sensor for Ag^+ in environment or organism.

Conclusion

In conclusion, we have synthesized a fluorescence turn-on probe **OA1** for Ag^+ detecting with 1,3,4-oxadiazole moiety as signal group and pyridine-2-formamidophenyl moiety as binding site. A remarkable enhancement (342 %) in fluorescent spectra with Ag^+ addition could be observed, and assays demonstrated this probe was specifically selective and sensitive for Ag^+ over competing cations in HEPES buffer solution (10 mM, pH=7.0, DMF-H_2O, 9:1, v/v). During recognization, a stable 1:1 **OA1**-Ag^+ complex formed. Due to its excellent properties, **OA1** could be a viable candidate as fluorescent receptor for Ag^+ analysis.

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