Photo-controlled Zn^{2+} release system with dual binding-sites and turn-on fluorescence†

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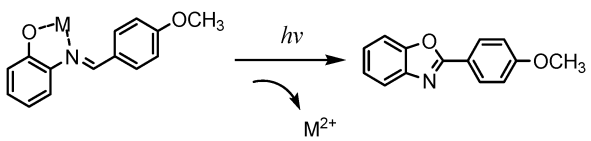
A phototriggered Zn-release system with dual binding-sites and turn-on fluorescence has been developed by employing a molecule with two Schiff base units as a ligand. It is found that the ligand with two Schiff base units can bind with two Zn^{2+}, and the bound Zn^{2+} can be released completely when the Schiff base is converted into bi-benzoxazole when phototriggered. During the conversion of the Schiff base into benzoxazole, the turn-on fluorescence was detected, which provides a convenient method to monitor the process of phototrigger release. Besides, it is also found that the amount of released Zn^{2+} depends on the exposure or irradiation time, which enables the possibility to manipulate the amount of Zn^{2+}-release *via* controlling irradiation time.

1. Introduction

The photo-controlled delivery of chemical and/or biochemical species offers great opportunities in photodynamic therapy,[1,2] chemical synthesis[3–5] and the characterization of reactive intermediates.[6–9] The light-activated release of metal ions has attracted considerable interest because some biologically active metals make an essential cofactor in numerous enzymes that are critical for life.[10–12] Zinc, for example, is an indispensable element in biology and free Zn^{2+} has been shown to influence a variety of receptors and ion channels present on cell surfaces, including NMDA[13] and GABA[14] receptors. Recent advances have shed light on the biological roles of zinc, particularly on its functions related to neurobiology.[15] It is believed that disorder of zinc homeostasis is implicated in a number of diseases,[16] such as Alzheimer's disease. The regulation of concentration of Zn^{2+} may also play a role in the control of Alzheimer's disease and excitotoxicity.[17,18]

The ability to control drug delivery in terms of quantity, location, and time is a key goal for drug delivery science, as improved control maximizes therapeutic effect while minimizing side effects.[19] The level of control which can be exerted on light delivered to a molecule in terms of wavelength, duration, intensity, and location can be exploited through a light-controlled drug liberation reaction to give control of the quantity of drug released, the timing of the release event, and its location. A fluorescent photo-controlled drug release system is useful because the fluorescence during *in vitro* experiments can be monitored to determine how much drug the system is likely to release.[20]

We have recently developed a phototriggered metal-ion release system[21] using a phenolic Schiff base derivative as ligand, in which the metal-ion is released when the phenolic Schiff base is converted into 2-arylbenzoxazole in the presence of NaOH by the phototrigger (Scheme 1). In addition, the latter compound has a diminished affinity for metal ions. In this paper, we expand our studies for two purposes: one is to extend the phototriggered release system from single binding-site to double binding-site, which enables metal-binding and metal-release in high concentration; and the other is to elucidate the fluorescence changes during phototrigger metal release, which may provide a convenient method to monitor the process of phototriggered release. In addition, we also found that the photoconversion of phenolic Schiff base to benzoxazole could be performed in the absence of base (NaOH), which is beneficial for applications in a physiological pH window. We employ a new phenolic Schiff base with dual binding-sites, **1**, as ligand (Scheme 2), and we found that the dual binding-sites bound to two Zn^{2+} to form a complex, and the bound Zn^{2+} could be released completely by a phototrigger in the absence of base. Moreover, the turn-on fluorescence was detected during the process of Zn^{2+}-release from the complex by a phototrigger. This probably provides a way for the control and determination of metal release by monitoring the fluorescence.



Scheme 1 Outline of the phototriggered metal-release system with single binding-site.

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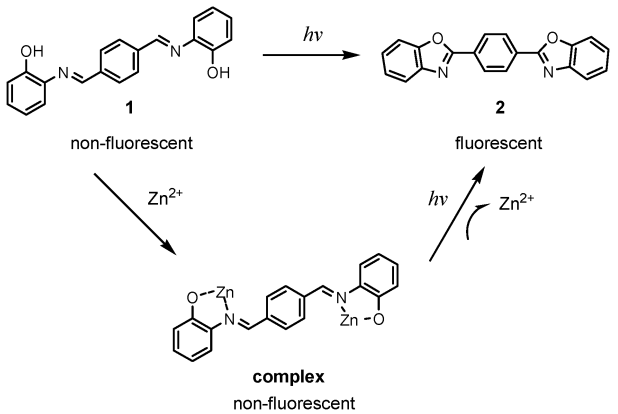
† Electronic supplementary information (ESI) available: Absorption changes of the photoreaction process of **2** and both absorption and fluorescence spectra for the control experiments of **2** binding with Zn^{2+}. See DOI: 10.1039/b917948h



Scheme 2 Outline of the phototriggered Zn^{2+}-release system with dual binding-sites.

2. Experimental

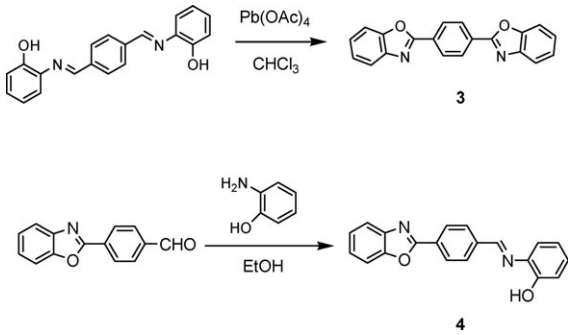
General

^{1}H NMR spectra were recorded at 400 MHz with TMS as an internal reference and $CDCl_3$ as solvent. UV absorption spectra were measured with an absorption spectrophotometer (Hitachi U-3010). All chemicals for synthesis were purchased from commercial suppliers, and solvents were purified according to standard procedures. Reactions were monitored by TLC silica gel plates (60F-254). Column chromatography was performed on silica gel (Merck, 70–230 mesh). A lamp with 365 nm (30 W) light was used as the light source for photoreactions.

Material

Ligand **1** was prepared by the general method described in the literature,[22] and the detailed procedure is presented as follows: terephthalaldehyde (6.7 g, 50 mmol) in ethanol (30 ml) was added to a solution of 2-aminophenol (11 g, 100 mmol) in ethanol (30 ml). The solution mixture was refluxed. When no starting material (terephthalaldehyde) was detectable by TLC plate, the mixture was cooled, and the crude product removed by filtration. Recrystallisation from n-butanol gave ligand **1** in 78% yield (12.3 g). mp = 221–223 °C (lit.[22] 220–221 °C). ^{1}H NMR ($CDCl_3$): 8.76 (s, 2H), 8.03 (s, 4H), 7.36 (d, J = 8.0 Hz, 2H), 7.26–7.21 (m, 4H), 7.05 (d, J = 8.1, 2H), 6.93 (t, J_1 = 8.0 Hz, J_2 = 8.0 Hz, 2H).

3 and **4** (2-((4-(benzo[d]oxazol-2-yl)methyleneamino)-phenol) were prepared according to the literature[23] and are illustrated in Scheme 3, and the detailed procedures and spectra data were as follows: a mixture of 2-((E)-((E)-4-((E)-(2-hydroxyphenylimino)methyl)benzyli-dene)amino)phenol (158 mg, 0.5 mmol) and lead acetate, $Pb(OAc)_4$ (244 mg, 0.55 mmol), dissolved in 20 ml of $CHCl_3$ was refluxed for 2 h. The solution was filtered and the filtrate was extracted with CH_2Cl_2–H_2O (v/v = 1/1, 10 ml × 3). The combined organic solution was dried over Na_2SO_4. The resulting solution was concentrated and the crude product was purified by recrystallisation from ethyl benzoate to give **3** (bis-p-phenylene-2′-benzoxazole) in 10% yield (16.2 mg). mp = 354–356 °C (lit.[24] 354 °C). ^{1}H NMR ($CDCl_3$): 8.43 (s, 4H), 7.82 (t, 2H, J_1 = 4.9 Hz, J_2 = 4.1 Hz), 7.63 (t, 2H, J_1 = 4.2 Hz, J_2 = 4.9 Hz), 7.41–7.39 (m, 4H).



Scheme 3 Synthetic routes for the preparation of **3** and **4**.

A mixture of 4-(benzo[d]oxazol-2-yl) benzaldehyde[25] (223 mg, 1.0 mmol) and 2-aminophenol (120 mg, 1.1 mmol) dissolved in anhydrous EtOH (20 ml) was stirred at room temperature for 0.5 h. During this time, the color of the solution gradually changed from colorless to yellow-green, and a yellow precipitate was produced. The yellow product was filtered off and washed with EtOH. The crude product was purified by recrystallisation from hexane to give **4** (2-((4-(benzo[d]oxazol-2-yl)methyleneamino)phenol) in 78% yield (245 mg). ^{1}H NMR ($CDCl_3$): 8.77 (s, 1H), 8.39 (d, 2H, J = 8.24 Hz), 8.08 (d, 2H, J = 8.3 Hz), 7.81 (t, 1H, J_1 = 4.9 Hz, J_2 = 4.0 Hz), 7.62 (t, 1H, J_1 = 4.1 Hz, J_2 = 4.9 Hz), 7.41–7.33 (m, 3H), 7.24 (t, 2H, J_1 = 7.2 Hz, J_2 = 7.4 Hz), 7.06 (d, 1H, J = 7.9 Hz), 6.93 (t, 1H, (t, 1H, J_1 = 7.9 Hz, J_2 = 7.3 Hz).

3. Results and discussion

The absorption changes of ligand **1** (λ_{max} = 387 nm, ε = 3.1 × 10^4 L mol^{-1} cm^{-1}, in CH_3CN) with the addition of Zn^{2+} were presented in Fig. 1. The absorption at 387 nm decreased, whilst a new band at 465 nm appeared, which corresponded to the absorption of the complex of ligand **1** binding to $Zn(OAc)_2$. The optical density of the complex is not increased until a stoichiometric amount of Zn^{2+} (1.0 equiv.) is added to the solution, which reveals the formation of a 1 : 1 complex. This formation is confirmed by the plot of the optical density of the complex against the amount of Zn^{2+} (inset in Fig. 1) according to the literature method,[26,27] and a binding constant (K_b = 3.3 × 10^3 M^{-1}) was estimated from the changes in the spectral intensities by using the Excel program.[28]

Upon 365 nm light irradiation, the absorption of the complex at 465 nm and 387 nm decreased and disappeared with increase of irradiation time and, accompanying this process, three new bands at 353, 336, 322 nm, respectively, which were attributed to the absorption of **2**, appeared (Fig. 2). Comparing the absorption of **2** with that of **3**, which was prepared according to the literature[23] and was illustrated in the experimental, it was found that the absorption spectra of **2** was similar to that of **3** (inset in Fig. 2), and absorption bands for both were exactly at 353, 336, 322 nm, respectively. As presented in Fig. 2, the absorption at 465 nm and 387 nm disappeared completely when the solution of the complex was

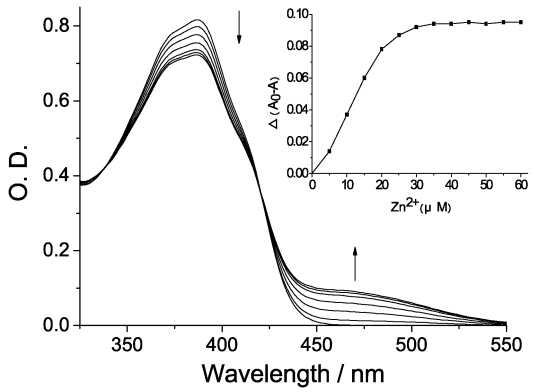


Fig. 1 Titration of 25 μM of ligand **1** with $Zn(OAc)_2$ in MeCN (periods: 0, 5, 10, 15, 20, 25, 30 μM). The inset describes a 1 : 1 binding isotherm with error bars calculated from three trials.

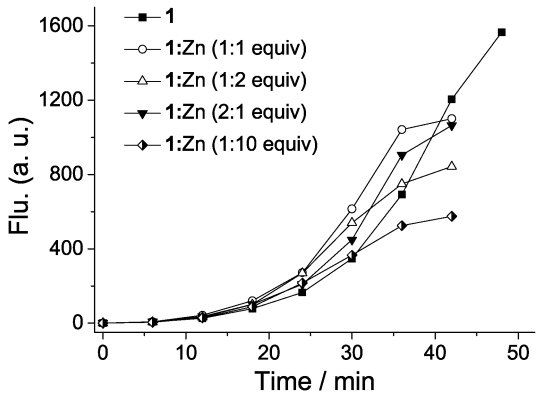


Fig. 3 Plot of fluorescence changes of **1** and the complex [**1** : Zn^{2+} (1 : 1 equiv.); **1** : Zn^{2+} (1 : 2 equiv.); **1** : Zn^{2+} (2 : 1 equiv.); and **1** : Zn^{2+} (1 : 10 equiv.)] against irradiation time (**1** : 25 μM, MeCN), λ_{ex} = 353 nm.

irradiated for 42 min. This suggested that Zn^{2+} was completely released during the conversion of the complex to **2**. It worth noting that this current photo-triggered release system could not be performed in pure water because ligand **1** did not dissolve in water, and it has not been carried out yet in other organic solvents, such as DMSO and EtOH or the mixtures of water with organic solvents.

The photoreaction process of **2** probably occurred *via* two routes. One is from both ligand **1** and the complex. Upon irradiation with 365 nm light, both absorption at 465 nm (absorption of the complex) and 387 nm (absorption of ligand **1**) decreased simultaneously, and both absorptions disappeared with increase of irradiation time. Another is from ligand **1** only. When ligand **1** photo-converted to **2**, the equilibrium between ligand **1** and the complex was disturbed, and the complex had to release Zn^{2+} to rebuild the equilibrium, which resulted in complete Zn^{2+}-release from the complex when **1** converted completely to **2** (for absorption changes of **1** when photo-converted to **2**, see Fig. 1 in the ESI†). To confirm the photoreaction process of Zn release, a competition experiment was carried out. As presented in Fig. 3, it took about 48 min for free ligand **1** to be completely converted to **2**, which is longer than that of the complex; the latter took 42 min to complete conversion. It is thought that the main photoconversion process is a result of the direct conversion of the complex instead of free ligand **1** only.

The fluorescence of **2** during the process of Zn^{2+}-photo-release was investigated. It is found that no fluorescence of the complex was detected in MeCN when using 353 nm as the excitation wavelength. The fluorescence of **2** was, however, observed when the complex was converted to **2** by a phototrigger. As presented in Fig. 4, the fluorescence of **2** appeared and increased with increase of irradiation time, and the largest fluorescence intensity was obtained with 42 min irradiation. By that time, the conversion of the complex to **2** had completed. Comparing the fluorescence of **2** with that of **3** (quantum yield φ_f = 0.42, life time τ_f = 1.14 ns, in CH_3CN, using quinine sulfate in 1 M H_2SO_4 as a standard), we found that the profile of the emission spectra of **2** was similar to that of **3** (inset in Fig. 4), and both emission bands were at the same position (λ_{em} = 370, 387, 408 nm) with the 353 nm excitation wavelength. Control experiments showed that no binding between **2** and Zn^{2+} was detected (see Fig. 2 and 3 in ESI†).

To explore the mechanism of the Zn^{2+}-release process, **4** (Scheme 3) with a benzoxazole unit and one Schiff base unit was prepared as a model for control experiments. First, it is found that the absorption at 360 nm with a shoulder at

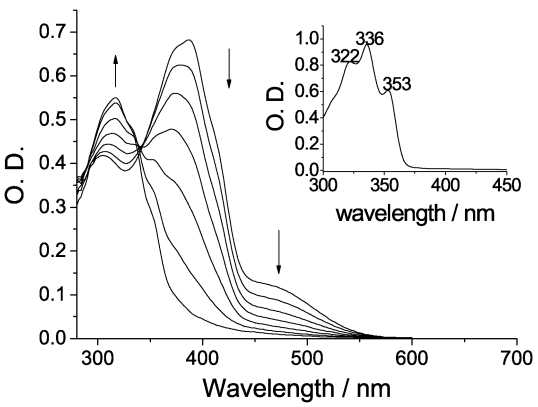


Fig. 2 Absorption changes of the complex upon 365 nm light irradiation (periods: 0, 7, 14, 21, 28, 35, 42 min). The inset shows the absorption of **3** (25 μM, in MeCN).

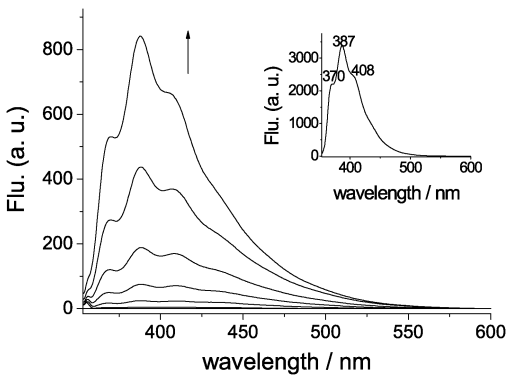


Fig. 4 Fluorescence changes of the complex upon 365 nm light irradiation (in CH_3CN, λ_{ex} = 353 nm, periods: 0, 7, 14, 21, 28, 35, 42 min). The inset shows the fluorescence of **3** (25 μM, in MeCN, λ_{ex} = 353 nm).

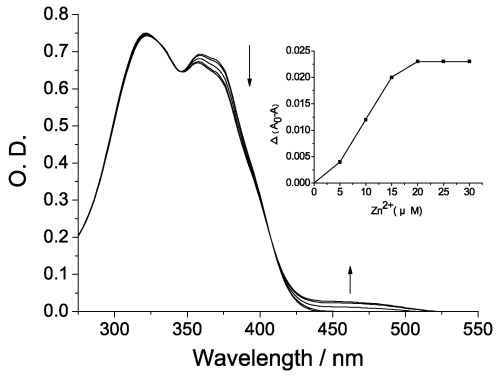


Fig. 5 Titration of 25 μM of **4** with $Zn(OAc)_2$ in MeCN. The inset describes a 2 : 1 binding isotherm with error bars calculated from three trials.

370 nm, which is attributed to **4**, decreases with the addition of Zn^{2+} and a new absorption at 455 nm, which corresponded to a 2 : 1 complex of **4** and Zn^{2+}, appeared (Fig. 5). Upon irradiation of the **4–Zn^{2+}** with 365 nm light, **4–Zn^{2+}** was completely converted to **2**. As shown in Fig. 6, the absorption of **2** from **4–Zn^{2+}** is same as the absorption of **3** (inset in Fig. 2). Second, **4–Zn^{2+}** showed no fluorescence emission with a 353 nm excitation wavelength, and a fluorescence (λ_{em} = 370, 387, 408 nm) was obtained only when the complex **4–Zn^{2+}** was converted completely to **2** (Fig. 7). Results indicated that two Zn^{2+} was released from the complex, instead of one during the process of Zn^{2+}-photorelease.

The kinetics of Zn^{2+}-photorelease with different irradiation times were also investigated by monitoring the change of the fluorescence of **2**. As presented in Fig. 8, the fluorescence intensity of **2** increased slowly for the first 20 min, and increased faster afterwards, during the process of Zn^{2+}-photorelease. It is likely that the emitted photons of **2** were re-absorbed by the complex due to considerable overlap between the absorption spectrum of the complex (λ_{max} = 387 nm) and fluorescence spectrum of **2** (λ_{em} = 387 nm). As the absorption band decreased and disappeared with irradiation time, this effect cancelled out, and the Zn^{2+}-release increased with irradiation time. The result shown in Fig. 8 indicated that the amount of Zn^{2+}-photorelease depends on irradiation time, and the quantity of Zn^{2+}-photorelease can be manipulated by controlling irradiation time.

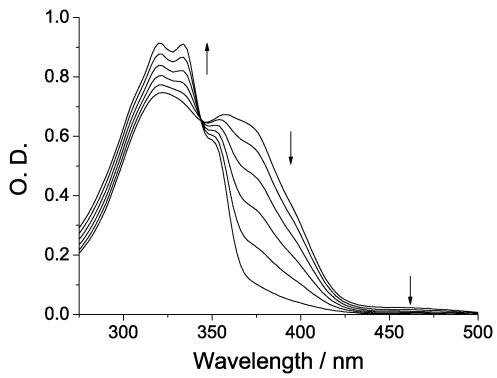


Fig. 6 Absorption changes of complex **4–Zn^{2+}** upon 365 nm light irradiation (periods: 0, 5, 10, 15, 20, 25 min).

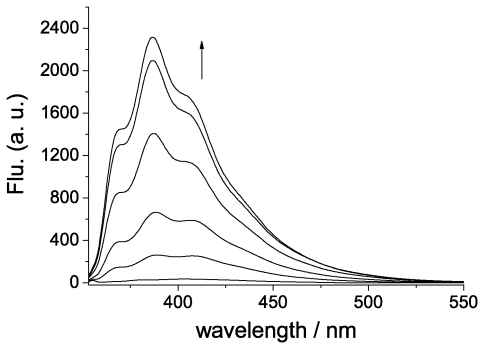


Fig. 7 Fluorescence changes of compound **4–Zn^{2+}** upon 365 nm light irradiation (periods: 0, 5, 10, 15, 20, 25 min, in CH_3CN, λ_{ex} = 353 nm).

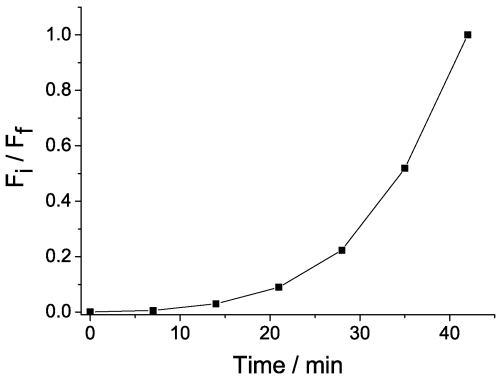


Fig. 8 Kinetics of Zn^{2+}-photorelease from the system (25 μM, in MeCN) with 365 nm light (30 W) at different irradiation times. The *y*-axis represents the initial integrated fluorescence response (F_i) over the final integrated emission (F_f).

This study represents a platform for the development of phototriggered metal release systems with dual binding-sites and fluorescence monitoring, which allow metal release in high concentrations and the process of metal release monitoring by fluorescence. Although the system presented herein has some shortcomings at present, such as the system not being able to be performed in aqueous solution, modification of the system is under way by covalently joining the Schiff base ligand to a water-soluble polymer or incorporating them in the interior of amphiphilic polymeric micelles. From a practical viewpoint, it is desirable for the system to have some merits, such as being performed in buffer solution and with visible light ($\lambda \geq 400$ nm) irradiation. This can be achieved by further modifying the molecular structure or environment and will be the subject of future studies.

4. Conclusion

In conclusion, the example presented herein clearly demonstrates that the metal release system with two Schiff base units can be converted into bis-benzoxazole by a phototrigger, which allows metal-binding and metal-release in high concentration. The results also demonstrated that the process of release can be monitored by fluorescence and the amount of metal-release can be manipulated by controlling

the irradiation time. Future studies will take advantage of the demonstrated system with photo-controlled release and turn-on fluorescence, and explore photorelease systems with multiplex binding-sites and nonlinear optical properties, which enable the possibilities of metal release in larger concentrations and three-dimensional distribution of release.

Acknowledgements

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