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Large Fluorescence Response by Alcohol from a Bis(benzoxazole)-Zinc(II) Complex: The Role of Excited State Intramolecular Proton Transfer

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Abstract

The formation of a bis(HBO) anion is known to turn-on the fluorescence to give red emission, via controlling the excited-state intramolecular proton transfer (ESIPT). The poor stability of the formed anion, however, hampered its application. The anion stability is found to be greatly improved by attaching the anion to Zn^{2+} cation (i.e. forming zinc complex), whose emission is at $\lambda_{\text{em}} \approx 550$ and 760 nm. Interestingly, addition of methanol to the zinc complex induces a remarkable red fluorescence ($\lambda_{\text{em}} \approx 630$ nm, $\phi_{\text{fl}} \approx 0.8$). With the aid of spectroscopic studies (^1H NMR, UV-vis, fluorescence, and mass spectra), the structures of the zinc complexes are characterized. The emission species is identified as a dimer-like structure. The study thus reveals an effective fluorescence switching mechanism that could further advance the application of ESIPT-based sensors.

Keywords

proton transfer; fluorescence; zinc complex; chemical sensor

Introduction

Due to its unique photophysical properties, excited-state intramolecular proton transfer (ESIPT) is emerging as a new design principle for fluorescent chemical sensors.^{1,2} In sharp contrast to most of other organic chromophores, ESIPT molecules exhibit large Stokes shift (ca. 150–200 nm, or 66667–50000 cm^{-1}), making them the ideal candidates for fluorescent sensors. As an important member of ESIPT-based molecules, 2-(2'-hydroxyphenyl)benzoxazole (HBO, **1**) has been reported for cation^{3,4,5,6,7} and anion^{8,9} sensing. Under the photon irradiation, HBO can generate both the excited *enol* (E^*) and *keto* (K^*) tautomers,^{3,7,4} whose emission are well separated from each other (e.g. $\lambda_{\text{em}} \approx 375$ nm for **1a**, and $\lambda_{\text{em}} \approx 500$ nm for **1b**). The overall emission property is dependent on the ratio of *enol/keto* tautomers. As a consequence, the sensor's performance is influenced by the environments such as solvents and temperature,¹⁰ as well as solvent polarity.¹¹ For HBO **1**, the *enol* emission at the short wavelength (~375 nm) typically increases with the solvent polarity,^{10,12,13} as hydrogen bonding of a polar solvent (e.g. EtOH or CH_3CN) with the phenol retards the proton transfer. In order to achieve the optimum performance of an ESIPT-based sensor, it is important to identify and understand the chemical events that can effectively control the emission between the *enol* and *keto* tautomers.

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Supporting Information Available: Spectral characterization of complexes **5** and **8**. This information is available free of charge via the Internet at <http://pubs.acs.org>.

2,5-Bis(benzoxazol-2'-yl)benzene-1,4-diol derivatives, such as bis(HBO) **2**, represents another interesting system, in which two HBO units are linked together. The derivatives of bis(HBO) have exhibited unique optical properties for high-contrast photochromic switching,¹⁴ and for two band emissions in sol-gel films.¹⁵ Our recent study shows that addition of an anion (e.g. OH⁻, F⁻, or OAc⁻) to **2** can increase the red emission of bis(HBO) by up to 20 fold.¹⁶ On the basis of the diminished Stokes' shift in **3** (in comparison with that in **2**), the nature of the anion-induced fluorescence turn-on is assumed to be emission from the *enol* tautomer of the anion **3**. In other words, addition of a base to **2** drastically shifts the absorption peak to a longer wavelength (by up to 100 nm depending on the counterion),¹⁶ while turning-on strong *enol* emission by formation of **3**. The anion-induced fluorescent species from bis(HBO) **2**, however, has poor stability (see discussion later), which limits its application.

By introduction of a zinc-chelating group in bis(HBO), we have also showed that weakly fluorescent **4** (Zinhbo-5, λ_{em} =600 nm) can form zinc complex such as **8**, in which the negative charge on the phenoxide is partially held by Zn²⁺ cation.¹⁷ As a consequence, the zinc complex **8** gives two stronger emission peaks: $\lambda_{em} \approx 550$ nm (from its *enol* form), and $\lambda_{em} \approx 760$ nm (from its *keto* form, ESIPT). The large spectral response upon zinc binding, in addition to its dual emission (with one in the near infrared region), makes the zinc complex a useful probe for Zn²⁺ detection.^{17,18} The crystal structure of **8** also revealed that the Zn–N(2) distance (1.987 Å) was notably shorter than Zn–N(3) (2.296 Å) and Zn–N(4) (2.046 Å), indicating the existence of a bonding between zinc and benzoxazole nitrogen. While the sensor **8** is thermodynamically favored product, the initially formed zinc complex upon mixing **4** and Zn²⁺ could have a different structure.¹⁸ We now demonstrate that the metastable zinc complex **5** can bind an alcohol to exhibit an unexpected bright red fluorescence turn-on with a stable red emission ($\lambda_{em} = 630$ nm, quantum yield $\phi_f \approx 0.8$). In comparison with the instability of **3**, the study demonstrates that the electronic property of the remaining HBO in bis(HBO) **5** can be tuned effectively through the connected zinc chelation center, thereby providing a new switching mechanism for ESIPT-based sensor.

Experimental Section

Instrumentation

Electrospray ionization (ESI) mass spectra were acquired with a Waters Synapt HDMS quadrupole/time-of-flight (Q/ToF) mass spectrometer. This instrument contains a triwave device located between Q and ToF mass analyzers and the device consists of three components: a trap cell, an ion mobility cell, and a transfer cell. The TWIM-MS experiments were performed with the following parameters: ESI capillary voltage, 3.5 kV; sample cone voltage, 30 V; extraction cone voltage, 3.2 V; desolvation gas flow, 550 L/h (N₂); trap collision energy (CE), 4.5 eV; transfer CE, 3.5 eV; trap gas flow, 1.5 mL/min (Ar); IM gas flow, 22.7 mL/min (N₂); sample flow rate, 10 μ L/min; source temperature, 35°C; desolvation temperature, 80°C; IM traveling wave velocity, 350 m/s; and IM traveling wave height, 8.5 V. The sample solution was sprayed with methanol.

Zinhbo-5 was prepared by using the reported procedure.¹⁸ All the solvents for the fluorescence experiments were analytic grade, which were purchased from Fisher Scientific and used without further purification. UV-Vis spectra were acquired on a Hewlett-Packard 8453 diode-array spectrometer. Fluorescence spectra were obtained on a HORIBA Jobin Yvon NanoLog spectrometer. The fluorescence quantum yields were obtained using quinine sulfate as the standard ($\phi_f = 0.53$, 0.1 M H₂SO₄). Zinhbo-5 was dissolved in dichloromethane (DCM) at the concentration of about 1×10^{-6} M (Excited at 412 nm for the fluorescence spectra). Then the Zn(OAc)₂ (in saturated DCM solution) was added dropwise

to form Zinhbo-5- Zn^{2+} complex, while the process was monitored by using UV-vis spectrometry. The fluorescence spectrum of Zinhbo-5- Zn^{2+} complex was obtained by excitation at 480 nm. Bright red fluorescence turn-on was obtained by addition of 20% MeOH (20% volume of DCM) to the Zinhbo-5- Zn^{2+} complex (Excited at 472 nm for the fluorescence spectra).

Results and Discussion

Stability of bis(HBO) anion **3**

UV-vis of **2** in THF ($1.71 \times 10^{-5} \text{M}$, 2.5 mL in a quartz cell) revealed two absorption peaks at 392 nm and 412 nm. Upon addition of LiOH solution in EtOH ($5 \times 10^{-1} \text{M}$, 5.0 μL), the absorption spectra revealed a new peak at 482 nm, indicating the formation of monoanion **3** (Figure 1). Disappearance of the absorption peaks at 392 nm and 412 nm showed the complete conversion of **2** to **3**. The anion **3**, however, was gradually decaying over the a short period of time. Interestingly, the decrease of the absorption band at 482 nm was accompanied with the recurring absorption bands at 392 nm and 412 nm, suggesting that the instability of the anion was due to its protonation. Observation of the isobestic point at 429 nm indicates the formation of only one new species (i.e. **2**). The decay of the anion appeared to obey the first order reaction, as the reaction in the first 15 minutes was basically linear (Figure 2b).

Absorption and fluorescence of Zinhbo-5- Zn^{2+} complex

The optical instability of bis(HBO) anion **3** led us to examine the Zinhbo-5- Zn^{2+} complex **5**, in which the negative charge on the phenoxide is partially held by Zn^{2+} cation. The solution of Zinhbo-5 exhibited the absorption $\lambda_{\text{max}}=413 \text{ nm}$ (Figure 2a), which was also observed in bis(HBO) **2** (Figure 1). Upon addition of Zn^{2+} cation, the absorption of Zinhbo-5 was shifted to $\lambda_{\text{max}}=480 \text{ nm}$, as a consequence of removing the phenolic proton ($\text{Ar-OH} \rightarrow \text{Ar-O}^- \text{Zn}^{2+}$). Similar absorption λ_{max} indicates that the phenoxide in Zinhbo-5- Zn^{2+} (complex **5**) has similar amount of negative charge on the phenoxide as mono anion **3**. The absorbance of the zinc complex **6** from "Zinhbo-5 + Zn^{2+} + ROH" did not change during the time period examined (ROH=MeOH or EtOH), in sharp contrast to that from **3** (Figure 2b). The improved stability can be attributed, at least in part, to the stronger bonding between PhO^- and Zn^{2+} (in comparison with PhO^- and Li^+ bond in **3**).

The solution of Zinhbo-5 in CH_2Cl_2 ($1 \times 10^{-6} \text{M}$) gave very weak fluorescence, which turned into stronger green fluorescence upon addition of one equivalent Zn^{2+} (Figure 3), as a consequence of the zinc complex formation.^{17,18} Interestingly, when a small amount of methanol was added on the top of "Zinhbo-5 + Zn^{2+} " solution, the bright red fluorescence was immediately observed at the interface between the methanol and CH_2Cl_2 layers (Figure 3c). And the intense red fluorescence was persistent when using a mixture of the methanol (~10–100%) and CH_2Cl_2 . Similar effect was also observed from ethanol. The results thus showed an intriguing fluorescence turn-on by an alcohol (methanol or ethanol), revealing a unique feature from the zinc complex **5**.

The fluorescence spectrum of "Zinhbo-5 + Zn^{2+} " revealed two characteristic emission peaks at 556 nm and 745 nm, attributing to the *enol* and *keto* emission of the zinc complex **5**, respectively (Figure 4).¹⁸ Addition of methanol induced a new intense emission band at 630 nm. By using quinine sulfate as standard, the quantum yield of the methanol adduct **6** was estimated to be ~81%. The absence of the near infrared (NIR) emission at ~745 nm suggests that the added alcohol turned off *ESIPT* emission (from the *keto* tautomer).

As to the nature of emission, the fluorescence peak of Zinhbo-5 at 600 nm was assigned to its *keto* tautomer arising from ESIPT, since the emission has a large Stokes' shift (600–413 = 187 nm, or 53417 cm⁻¹).¹⁶ The emission of “Zinhbo-5 + Zn²⁺ + MeOH” was occurring at 630 nm, which was between the *enol* (at 548 nm) and *keto* emission wavelength (at 745 nm) of “Zinhbo-5 + Zn²⁺”. It should be noticed that the *keto* emission from the known bis(HBO) chromophores (such as **2** & **4**) exhibit no overlap between the absorption and emission spectra.^{16,18,19} The slight spectral overlap between the absorption and emission at ~525 nm led us to think that the emission at 630 nm is likely to be from the *enol* tautomer of the methanol adduct **6**.

Study of Complex Structure by NMR and Mass Spectroscopy

In order to probe the nature of the alcohol binding to the metal complexes, we examined the ¹H NMR of Zinhbo-5 in CDCl₃. As seen in Figure 5, the characteristic pyridyl proton *H_a* (labeled in the Zinhbo-5 structure **4**) at ~8.5 ppm was shifted to ~8.9 ppm when Zinhbo-5 was binding to Zn²⁺ to form the complex **5**. The NMR titration also showed that the formed complex in CH₂Cl₂ had a 1:1 ligand-to-metal ratio. Addition of 2% methanol into the solution caused the proton signal *H_a* to shift slightly upfield to ~8.8 ppm, suggesting the weakening of the pyridyl–Zn²⁺ bonding. The added methanol, however, did not break the complexation bond ArO⁻–Zn²⁺, as the UV-vis absorption of “Zinhbo-5+Zn²⁺ + MeOH” solution revealed no trace of free ligand Zinhbo-5 in the solution (no characteristic absorption signal at 393 nm & 413 nm, see Figure 2a).

Two methylene protons *H_c* and *H_d* of Zinhbo-5 gave two singlet peaks at 4.1 and 3.9 ppm (in 1:2 ratio), which became a relative complicated pattern as a consequence of forming zinc complex **5**. Addition of methanol changed the *H_c* and *H_d* signals to two singlet peaks (at 4.3 and 4.2 ppm). On the basis of the experimental evidence, the methanol was assumed to interact with the Zn²⁺ binding site, possibly via the electrostatic attraction between the hydroxyl of alcohol and Zn²⁺ cation. It should be pointed out that the methanol binding interaction was not sufficiently strong to break the pyridyl–Zn²⁺ bonding, as the observed *H_c*' and *H_d*' signals (Figure 5f) are still quite different from that in the free ligand Zinhbo-5 (Figure 5a). In other words, the incoming alcohol was assumed to be attached to the Zn²⁺ site (as shown in **6**) without breaking the existing pyridyl–Zn²⁺ and PhO⁻–Zn²⁺ bonding, since the bonding of PhO⁻–Zn²⁺ was also kept intact in the presence of alcohol (Figure 2a).

Electrospray ionization (ESI) mass spectrometry was used to gain additional information about the complex structure. The solution of “Zinhbo-5 + Zn²⁺” in CH₂Cl₂ detected the exact mass of *m/z*=674.1741 (Figure 6a), corresponding to the zinc complex **5** (the calcd mass for C₃₇H₃₂N₅O₄Zn⁺: 674.1746). Upon addition of 20% methanol to the zinc complex solution, a doubly charged peak at *m/z* 705.1889 was detected (Figure 6b), indicating that the methanol adduct could induce a dimer structure shown as **6**.

Figure 7 shows two-dimensional ESI-traveling wave ion mobility mass spectrometry (TWIM-MS) plot²⁰ for *m/z* 674 and 706. The zinc complex {Zinhbo-5⁻+Zn²⁺} (**5**) gave the signal at drift time ~6.5 ms. Interestingly, the species formed upon addition of MeOH to **5** gave signal at a quite smaller drift time (~3.85 ms), which ruled out the formation of [Zinhbo-5⁻+Zn²⁺+MeOH]. Therefore, the new species is likely to be the dimer [Zinhbo-5⁻+Zn²⁺+MeOH]₂, which has the same *m/z* (=706). The assumption is consistent with its higher mass, whose ion mobility separation would give signals at different drift time (ms). The observation, in addition to the observed isotope pattern (Figure 6), thus confirmed that the dimer **8** was formed during the alcohol-induced fluorescence turn-on. The observation of *m/z* at 705 in Figure 6b suggests that some of the complex **6** could be

oxidized to **9**, by losing a hydrogen on the bound methanol which is accompanied by oxydation of phenol (Scheme 3).

The freshly prepared zinc complex **5** gave strong alcohol-induced fluorescence turn-on (Figure 3). If the solution of the zinc complex was left for overnight, however, the fluorescence turn-on was found to be much weaker. In order to further confirm the structure, the crystal sample **9** was used to repeat the experiment, whose complex structure has been determined by X-ray crystal structure.¹⁸ The solution of **9** in CH₂Cl₂ revealed two emission bands at about 534 and 750 nm (similar to that of **5**), attributed to the emission from its *enol* and *keto* tautomers, respectively. Interestingly, addition of 20% methanol (by volume) raised the *keto* emission without generating a new band at ~630 nm. When about 40% methanol was added, a shoulder started to form at about 635 nm, while the emission bands at 534 and 750 nm were decreased. The very different fluorescence response from **9** clearly indicated the zinc complex **5** must have a different structure, which is also evident from the ¹H NMR (ESI Figure S2 and S4). In light of the above spectroscopic evidences, we propose the formation of zinc complex **5**, in which the nitrogen atom on benzoxazole ring was not linked to Zn²⁺ cation, as doing so would lead to crystal sample **8**. In the proposed structure **5**, the phenoxide was bonded to Zn²⁺ cation as shown, on the basis of the observed absorption band at ~472 nm (Fig. 2a). The Zn²⁺ cation in the complex **5** has a coordination number of four, which is common for a zinc complex.

The remaining HBO unit of **6** could interact with additional methanol molecules, via intermolecular hydrogen bonding, to lead to the “open structure” isomer.^{21,22} This could result in the possible “open structures” **10** or **11** (Scheme 4), where the intramolecular hydrogen-bonding in one HBO unit (or both HBO) is open. It should be noted that the content of the “open structure” **10** or **11** may not be high, as intramolecular hydrogen bonding is stronger than the intermolecular one. The assumption appears to be consistent with the observation that the emission from the *keto* tautomer of HBO²³ and bis(HBO) **2**¹⁶ are predominant in a pure protic solvent such as methanol. Although the formation of the “open structure” in **10** or **11** could contribute to the “ESIPT-off” event, it can not explain the experimental observation that the 20% methanol can completely turn off ESIPT in **6** (Figure 4, Figure S11) but not in **8** (Figure 8). In addition, the formation of “open structure” should not dramatically shift the *enol* emission (λ_{em}~550 nm). On the basis of the available evidences, we assume the alcohol binding to the zinc center is responsible for the observed red fluorescence turn-on.

In conclusion, Zinhbo-5 has been found to initially form the tetra-coordinated zinc complex **5**, which has a 1:1 zinc-to-ligand ratio and give emission at 550 and 745 nm. In the complex **5**, the ESIPT emission is enabled, as Zn²⁺ cation partially hold the negative charge on the phenoxide. Upon addition of an alcohol (methanol or ethanol), the resulting complex **6** is found to have a dimer-like structure, which disabled ESIPT to give intense red fluorescence at ~630 nm (via enabling the *enol* emission). The study illustrates for the first time that bis(HBO)-zinc complex can generate a stable red-emission, in sharp contrast to the poor stability of bis(HBO) mono anion **3** (see Figure 2b).

In the complex **6**, the Zn²⁺ cation has a penta-coordination after one methanol is added to the Zn²⁺ ion. Different from the simple solvent effect, the adduct appears to have a defined structure, whose formation allows the turn-on of red fluorescence. The sequence of the event thus can be described in Scheme 2. When methanol was added to “Zinhbo-5--Zn²⁺” complex, the positive charge on Zn²⁺ cation is partially delocalized to the bound methanol in **6**. As a consequence, the Zn--OAr bond became longer, which resulted in the fluorescence turn-on of *enol* emission, giving enhanced red emission. The alcohol binding-enabled fluorescence turn-on thus provides a new mechanism for sensor applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. UV-vis of **2** (dotted line) and its mono anion **3** (solid line) in the mixture of THF and EtOH (30:1). The spectra of **3** were taken after mixing **2** and LiOH for different times.

Figure 2.

(a) UV-vis absorption of Zinhbo-5 in CH_2Cl_2 , upon subsequent addition of equimolar Zn^{2+} and 20% methanol. (b) Stability of “Zinhbo-5+ Zn^{2+} ” in the presence of methanol (solid line) by monitoring the absorbance at ~472 nm, in comparison with the stability of anion **3** (dotted line, data from Figure 1).

Figure 3.

Fluorescence images of Zinhbo-5 (a) and Zinhbo-5+Zn²⁺ (1:1 ratio) in CH₂Cl₂ (c), when the solution was irradiated by using a fluorescent lamp (364 nm) from top. (d) A small amount of methanol was placed at the top of “Zinhbo-5+Zn²⁺ CH₂Cl₂” solution. (e) Zinhbo-5+Zn²⁺ was in the mixed solvent (20% methanol in CH₂Cl₂). (b) Crystal **8** (Zinhbo-5+Zn²⁺) in the mixed solvent (20% methanol in CH₂Cl₂).

Figure 4. Fluorescence spectra of Zinhbo-5 in CH_2Cl_2 , upon subsequent addition of Zn^{2+} and 20% methanol at room temperature.

Figure 5. ^1H NMR of Zinhbo-5 in CDCl_3 , upon addition of different amount of Zn^{2+} cation (b–e), followed by 2% deuterated methanol (f).

Zinhbo-5 + Zn(OAc)₂

Zinhbo-5 + Zn(OAc)₂ + MeOH

Figure 6. ESI-MS spectra of “Zinhbo-5 + Zn²⁺” (a) and “Zinhbo-5 + Zn²⁺ + MeOH” (b). The experimental isotope patterns match the theoretical prediction (bottom spectrum c).

Figure 7.

Two-dimensional ESI-TWIM-MS plot for “Zinhbo-5 + Zn^{2+} + MeOH,” which was isolated by quadrupole at a traveling wave height of 8.5 V and velocity of 350 m/s. Ion mobility separation gave signals at different time (ms), corresponding to $[\text{Zinhbo-5} + \text{Zn}^{2+}]$ at $m/z=674$, and $2[\text{Zinhbo-5} + \text{Zn}^{2+} + \text{MeOH}]$ at $m/z=706$, respectively.

Figure 8. Fluorescence spectra of crystal **8** in CH_2Cl_2 upon addition of different amount of MeOH.

Scheme 1.
Structures of HBO and bis(HBO).

Scheme 2.

Structures of Zinhbo-5 and its zinc complexes, where R-OH is CH₃OH or C₂H₅OH.

Scheme 3.

Possible formation of $m/z = 705$ peak in the mass spectrum of Figure 6, when the molecule is travelling to the detector.

Scheme 4.

Methanol interaction with HBO to give possible "open structures" **10** and **11**.