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PAPER

Mononuclear Zn(II)- and Cu(II)-complexes of a hydroxynaphthalene-derived dipicolylamine: fluorescent sensing behaviours toward pyrophosphate ions†

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Mononuclear Zn(II)-DPA and Cu(II)-DPA complexes crafted on 2-hydroxy-6-cyanonaphthalene fluorophore selectively recognize PPi over ATP and other anions including inorganic phosphates in aqueous medium, showing turn-on type fluorescence enhancements. Coordination of a hydroxyl group of the fluorophore, directly or in alkoxy form, to the central metal ion is crucial for the sensing processes. Both the complexes elicit a fluorescence increase in a time-dependent fashion.

Introduction

Anions play important roles in various chemical and biological processes. Accordingly, there has been considerable interest in sensing systems for anionic species.[1] Among various methods for detecting anions, the fluorescence detection method has received significant interest owing to its operational simplicity and high sensitivity. Thus, anion-selective fluorescent probes[2,3] and colorimetric probes[2] have attracted increasing attention. Among the target anions, pyrophosphate (PPi) has attracted much attention because it is the product of ATP hydrolysis under cellular conditions.[4] The detection of pyrophosphate was investigated as a real-time DNA sequencing method.[5] The detection of PPi has also become an important issue in cancer research.[6] Patients with calcium pyrophosphate dehydrate crystals and chondrocalcinosis have a high synovial fluid PPi level.[7] Abnormal PPi levels can lead to vascular calcification, which results in severe medical conditions.[8]

Since Czarnik's pioneering work in fluorescent sensing of PPi using a polyamine-attached anthracene derivative in aqueous medium,[9] considerable effort has been made to develop molecular probes for the optical detection of PPi.[10] In the development of PPi probes, desirable features are the selective detection of PPi over related analogues such as ATP, ADP and other inorganic phosphates (Pi) in aqueous medium. Also realization of a "turn-on" type fluorescence response in the presence of the analyte is preferred over a "turn-off" response, as the former provides a better sensitivity over the latter.

So far, receptors comprising of two binding sites of zinc or copper complexes have been widely used in devising fluorescent probes for PPi.[11] With such receptors, analyte recognition is favoured by cooperative interactions. In addition, carefully designed receptors can distinguish between PPi and ATP because they have different total anionic charge densities.[12] Recently a few mononuclear Cu(II)-dipicolylamine (DPA) complexes were reported, which sense PPi selectively.[13] Such mononuclear metal complexes are relatively unexplored compared to the dinuclear complexes. Thus, we planned to study mononuclear metal complexes such as **1**·Zn(II) and **1**·Cu(II) that are crafted onto 2-hydroxy-6-cyanonaphthalene, an electron "push-pull" type fluorophore (Fig. 1). Some push-pull type fluorophores show interesting photophysical behaviours such as two-photon absorbing and environment-sensitive properties.[14] We reasoned that the 2-hydroxyl group of the fluorophore can coordinate to the metal ion bound to the appended DPA ligand.

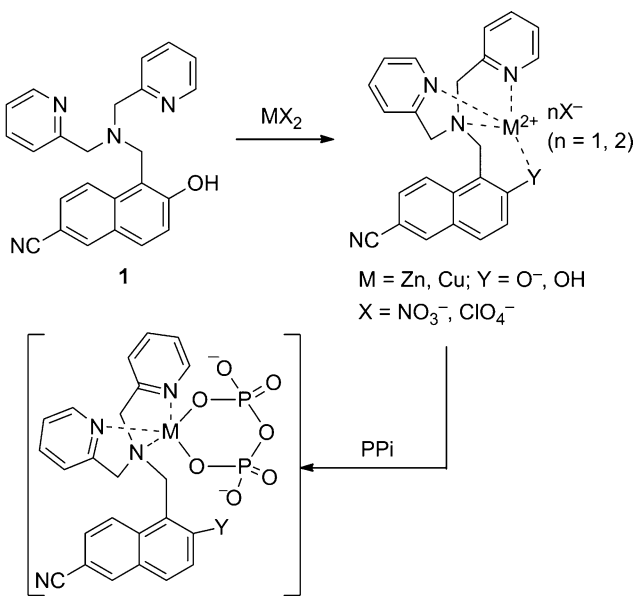

Fig. 1 Schematic presentation of the Zn(II) and Cu(II) complexes of dipicolylamine–hydroxynaphthalene **1**, and their PPi-bound complexes.

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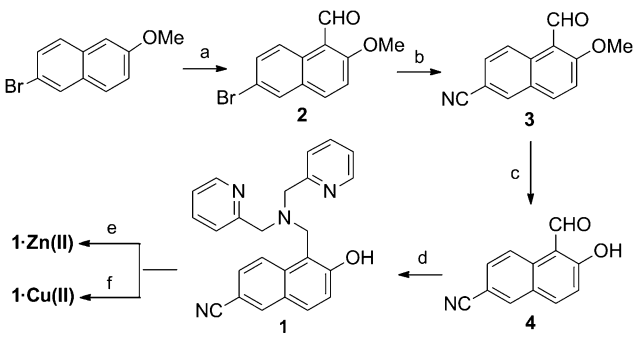
This coordination, in-turn, may perturb the electronic push-pull event of the naphthalene fluorophore. The metal coordination may reduce the electron density over the hydroxyl (or alkoxy) site.

When PPi binds to the central metal ion, the coordinated hydroxyl group may be released, which restores the push-pull characteristics. Thus, a significant change in the fluorescence may be realized. Especially in the case of a Cu(II) complex, being a d^9 system, the ligand-to-metal charge transfer (LMCT) may occur, thus affecting the polarization of the fluorophore; such a charge transfer may be blocked upon binding PPi, which reduces the positive charge on the copper. Such coordination changes in the hydroxyl group are expected to accompany changes in the photophysical properties of the naphthalene fluorophore, enabling the optical sensing of PPi.

Results and discussion

On the basis of the above rationale, we synthesized the Zn(II)- and Cu(II)-complexes, **1**·Zn(II) and **1**·Cu(II) respectively, starting from commercially available 6-bromo-2-methoxynaphthalene. 6-Cyano-2-hydroxy-1-naphthaldehyde (**4**), prepared in three steps from the starting material, was treated with dipicolylamine under a reductive amination condition to furnish DPA **1**. The desired metal complexes were obtained by adding one equivalent of zinc and copper nitrate, respectively, to DPA **1** in methanol at room temperature (Scheme 1). These metal complexes were purified by recrystallization. Details of the synthesis and characterization of the compounds are described in the experimental section.



Scheme 1 Synthesis of DPA **1** and its metal complexes, **1**·Zn(II) and **1**·Cu(II). Reagent and conditions: (a) $TiCl_4$, Cl_2CHOMe, CH_2Cl_2, 0 °C–r.t., 10 h (80%); (b) CuCN, NMP, 135 °C, 8 h (60%); (c) BBr_3, CH_2Cl_2, 0 °C–r.t., 8 h (55%); (d) $NaBH(OAc)_3$, di(2-picolyl)amine, CH_2Cl_2, r.t., 8 h (40%); (e) $Zn(NO_3)_2·6H_2O$, MeOH, r.t. (81%); (f) $Cu(NO_3)_2·2.5H_2O$, MeOH, r.t. (85%).

The structures of **1**·Zn(II) and **1**·Cu(II) are given in Fig. 2. In the ^{1}H NMR spectrum of DPA **1**, the two methylene hydrogens adjacent to the naphthalene ring and the four methylene hydrogens adjacent to the two pyridine rings appeared as two sets of singlets at δ 4.26 and 3.91 ppm, respectively; upon complexation with zinc nitrate, the later methylene signals were split into an AB system at δ 4.34 and 4.16 ppm with a large coupling constant of 16.2 Hz (ESI†). In a high resolution mass spectrum recorded with **1**·Zn(II), the base peak appeared at 443.0853 ($C_{24}H_{19}N_4OZn$); this mass data suggests that the hydroxyl group of DPA **1** coordinates to the zinc ion in its alkoxy form (ESI†).

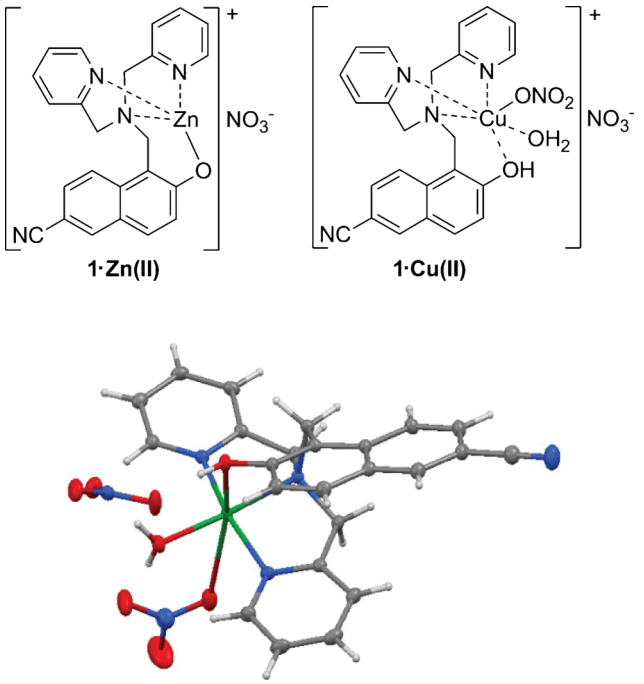


Fig. 2 Structures of Zn(II) and Cu(II) complexes of DPA **1**, **1**·Zn(II) and **1**·Cu(II), and an ORTEP plot of **1**·Cu(II) with 50% probability.

We observed time-dependent fluorescence behavior from the zinc complex, which also supported the formation of such a zinc–alkoxide bond (*vide infra*). In contrast, the hydroxyl group of DPA **1** coordinates to the copper ion directly, not in the form of the alkoxy group. The crystal structure of a Cu(II)-complex of DPA **1**, **1**·Cu(II), is given in Fig. 2.[15]

The coordination geometry of the central Cu(II) atom is distorted square pyramidal with three N atoms and one O atom of the coordinated water molecule in the equatorial plane, and the O atom of the hydroxyl group oriented axially. Two nitrate anions in the unit cell balance the formal charge (2+) of the central copper atom. The Cu–N bond distances range from 1.968(11) to 2.032(11) Å. The equatorial Cu–O_{water} distance is 1.965(10) Å and the axial Cu–$O_{(phenolate)}$ distance is 2.384(10) Å.[16]

Fluorescence change of **1**·Zn(II) was examined against various anions (200 equivalents; PPi, ATP, ADP, Pi (phosphate), AcO^-, HPO_4^{2-}, HSO_4^-, F^- and Cl^-) in a HEPES buffer (10 mM, pH 7.4).

The zinc complex **1**·Zn(II) shows a large fluorescence enhancement in the presence of PPi, followed by ATP, and others show little or no enhancement (Fig. 3). The fluorescence enhancement was saturated upon addition of 250 equivalents of PPi, showing about 17-fold enhancement (Fig. 4). In the course of our study, Yoon and co-workers also reported mononuclear Zn(II) and Cu(II) complexes of a coumarin-derived DPA ligand.[13b] In both complexes, the hydroxyl group participates in binding the metal ions in the same ways as in our cases; however, they show fluorescence behaviours different from our complexes toward PPi. Their zinc(II) complex does not show any fluorescence change toward PPi and other anions, whereas their copper(II) complex exhibits approximately 3-fold enhancement in the fluorescence intensity upon the addition of PPi.

Unexpectedly, we observed that our Zn(II)-DPA complex, **1**·Zn(II) elicits fluorescence enhancement with PPi in a

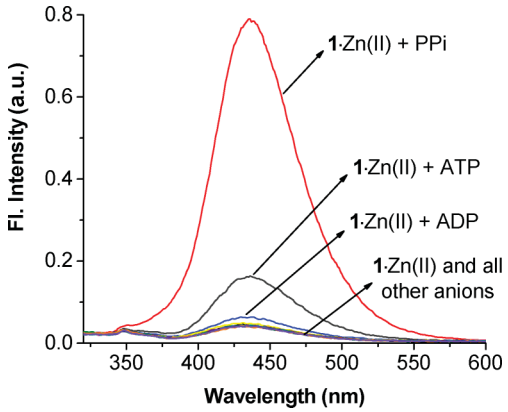


Fig. 3 Fluorescence response of **1**·Zn(II) (10 μM) toward each of various anions (200 equiv.; PPi, ATP, ADP, **1**·Zn(II), $H_2PO_4^{2-}$, AcO^-, PO_4^{3-}, HSO_4^-, ClO_4^-, F^-, Cl^-, Br^-, I^-, NO_2^- and NO_3^- respectively) as their sodium salts in a HEPES buffer (10 mM, pH 7.4); measured after 6 h upon addition of the anion (excitation at 310 nm; intensity was estimated by the peak height at $\lambda = 435$ nm).

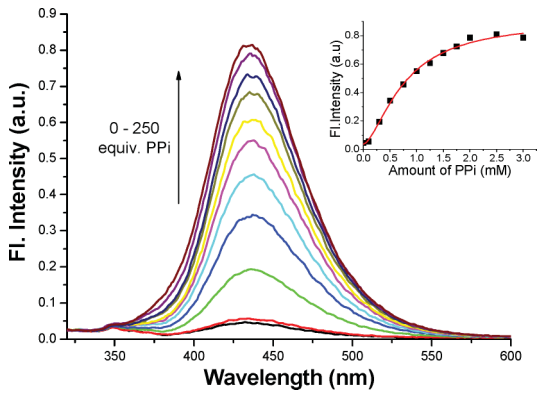


Fig. 4 Fluorescence titration of **1**·Zn(II) (10 μM) against PPi in 10 mM HEPES buffer of pH 7.4, measured 6 h after addition of analyte (excitation at 310 nm; intensity was estimated by the peak height at $\lambda = 435$ nm).

time-dependent fashion, as shown in Fig. 5. This behaviour may be attributed to a slow cleavage of the Zn–$O_{(phenolate)}$ bond as PPi binds to the Zn(II) ion. To investigate the time-dependent fluorescence change phenomenon, we synthesized DPA **5**, a methoxy analogue

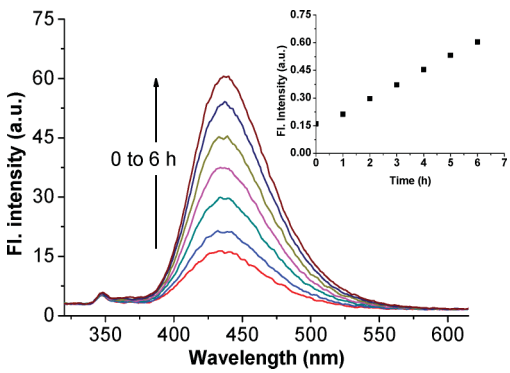
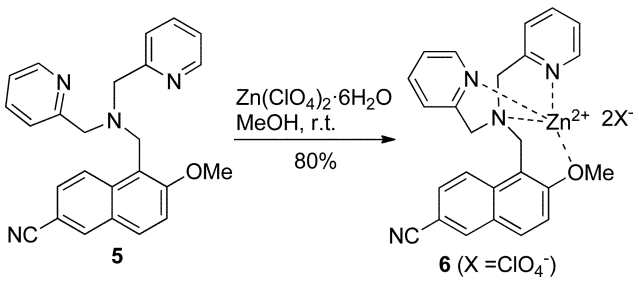


Fig. 5 Time-dependent fluorescence enhancement of **1**·Zn(II) (10 μM) in the presence of 100 equiv. of PPi in 10 mM HEPES buffer of pH 7.4 (excitation at 310 nm). Inset: a plot of fluorescence intensity change with time (intensity was estimated by the peak height at $\lambda = 435$ nm).

of DPA **1**, and its zinc complex **6** (Scheme 2). The zinc complex **6** does not show time-dependent fluorescence behaviour in presence of PPi (Fig. 6). We found that such time dependency was also independent of the counter anions (ESI†). The results support our explanation that a slow breaking of the Zn–$O_{(phenolate)}$ bond upon PPi binding is responsible for the time-dependent fluorescence behavior of **1**·Zn(II).



Scheme 2 Synthesis of Zn(II) complexes **6**.

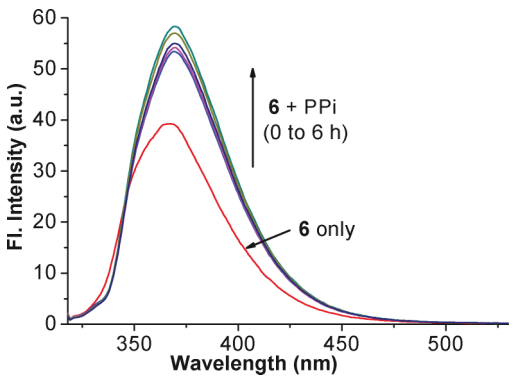


Fig. 6 Fluorescence enhancement of **6** (10 μM) in the presence of 100 equiv. of PPi in 10 mM HEPES buffer of pH 7.4 (excitation at 310 nm).

The quantum yield of PPi-bound **1**·Zn(II) was estimated to be 0.19 (200 equivalents of PPi; quinine sulfate in 0.1 M H_2SO_4 as reference) in a HEPES buffer (10 mM, pH 7.4). Mass peaks at 665.0 [$(C_{24}H_{21}N_4Na_2O_8P_2Zn)^+$ *i.e.* **1**·Zn(II) + $Na_2P_2O_6(OH)^-$] and 688.8 [$(C_{24}H_{20}N_4Na_3O_8P_2Zn^+)$ *i.e.* **1**·Zn(II) + $Na_3P_2O_7^-$] recorded in the electrospray (ES)-(+)-mode and also the peak at 618.2 (for $[C_{24}H_{21}N_4O_8P_2Zn]^-$ *i.e.* **1**·Zn(II) + $P_2O_6(OH)^{3-}$] recorded in the ES-(–)-mode correspond to the formation of a 1 : 1 adduct between **1**·Zn(II) and PPi. From the fluorescence titration data, the association constant between **1**·Zn(II) and PPi was determined to be 1×10^5 M^{-1}.[17]

Then, we evaluated fluorescent behaviors of **1**·Cu(II) toward various anions (900 equivalents; PPi, ATP, ADP, PO_4^{3-}, HPO_4^{2-}, AcO^-, Cl^-, F^-, I^-, Br^-, N_3^-, NO_2^-, NO_3^-, HSO_4^-, and ClO_4^-) in a HEPES buffer (10 mM, pH 7.4). It was observed that **1**·Cu(II) exhibits a turn-on fluorescence response only toward PPi selectively, and no response toward other anions (Fig. 7). From the fluorescence titration experiment, about 24-fold enhancement is obtained in the presence of excess PPi (Fig. 8), which is an eight times higher value than that observed by the coumarin-based Cu(II) complex reported by Yoon and co-workers.[13b] The fluorescence enhancement is possibly owing to the electrostatic interaction between PPi and **1**·Cu(II) in which two oxygen atoms

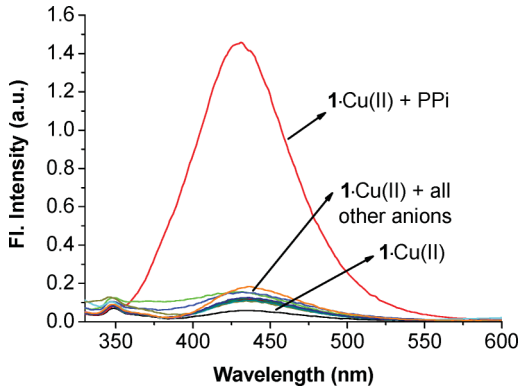


Fig. 7 Fluorescence response of **1**·Cu(II) (10 μM) toward each of various anions (900 equiv.; PPi, **1**·Cu(II), ATP, ADP, PO_4^{3-}, HPO_4^{2-}, AcO^-, F^-, I^-, Br^-, Cl^-, N_3^-, NO_2^-, NO_3^-, HSO_4^-, and ClO_4^- respectively) as their sodium salts in a HEPES buffer (10 mM, pH 7.4); measured after 6 h upon addition of the analyte (excitation at 325 nm; intensity was estimated by the peak height at $\lambda = 437$ nm).

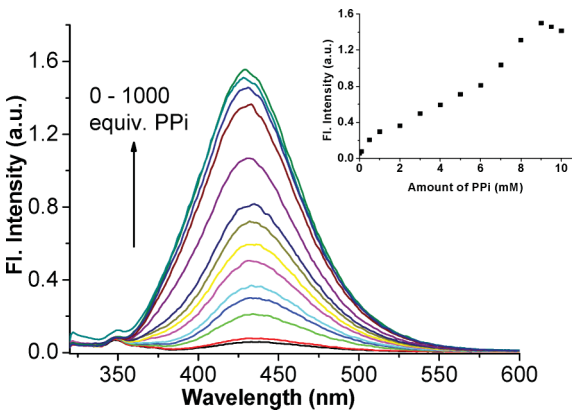


Fig. 8 Fluorescence titration of **1**·Cu(II) (10 μM) against PPi in 10 mM HEPES buffer of pH 7.4, measured 6 h after addition of the analyte (excitation at 325 nm). Inset: a plot of fluorescence intensity change against [PPi] (intensity was estimated by the peak height at $\lambda = 437$ nm).

of PPi coordinate with the central Cu^{2+} ion, reducing the charge density on the Cu^{2+} ion; hence, the ligand to metal charge transfer may be suppressed. It should be mentioned that a Cu(II) complex of DPA **5** does not show any fluorescence change in presence of PPi, ATP and other inorganic phosphates (ESI). This observation points to the significant role of the free hydroxyl group in the recognition process.

Contrary to our expectation, **1**·Cu(II) also showed a gradual increase in fluorescence with time in the presence of PPi (ESI†). The reason is not clear but a slow ligand exchange involving the coordinated water or the nitrate ligand may be responsible for the time-dependent fluorescence change.[18] The observed time-dependent fluorescence behaviors are not disclosed in the cases of reported coumarin-derived zinc and copper complexes;[13b] however, it seems that these complexes also show time-dependent behaviour because the PPi binding modes are expected to be the same as those of the present systems. In any case, the time-dependent behaviour should be kept in mind in designing such mononuclear metal complexes as PPi sensing systems.

The quantum yield of **1**·Cu(II) in the presence of excess PPi (900 equivalents) was estimated to be 0.12 (quinine sulfate in 0.1 M H_2SO_4 as a reference) in a HEPES buffer (10 mM, pH 7.4). The fluorescence titration data (inset of Fig. 8) shows a more complex behavior in comparison with that of the zinc complex (Fig. 4); it does not show a simple 1 : 1 binding mode. An excess amount of PPi (900 equivalents) resulted in highest enhancement; but after that point, quenching occurred. These results indicate that the copper complex binds PPi less tightly than does the zinc analogue.

Conclusions

We have studied two mononuclear Zn(II) and Cu(II) complexes based on a dipicolylamine ligand crafted onto a naphthol derivative of a push-pull fluorophore. Both of these complexes show weak fluorescence but exhibit turn-on response only in the presence of pyrophosphate among other anions including phosphate examined. The zinc and copper complexes selectively recognize PPi over ATP in aqueous medium with 17-fold and 24-fold enhancements in the fluorescence intensity, respectively. Both of these complexes elicit a fluorescence increase in a time-dependent fashion, suggesting slow ligand exchange in both cases. The copper complex is characterized by single crystal X-ray crystallography. The fluorescence behavior observed in this study provides an insight for the development of related metal complexes for the fluorescent sensing of PPi.

Experimental

General methods

Chemicals from Sigma-Aldrich were used without further purification. All reactions were performed under argon atmosphere unless otherwise stated. Analytical TLC was performed on Merck silica gel (60 F254) plates (0.25 mm) and visualized with ultraviolet light. ^1H and ^{13}C NMR spectra were measured with a Bruker DPX-300 spectrometer. Coupling constants (J value) are reported in Hertz. The chemical shifts are shown in ppm. UV/Vis absorption spectra were obtained using a HP 8453 UV/Vis spectrophotometer. FTIR spectra were recorded at a spectral resolution of 4 cm^{-1} with a BRUKER VERTEX 70 spectrometer. High-resolution mass spectra were recorded on a JEOL JMS-700 spectrometer. Melting points were measured using an electrothermal MELT-TEMP 3.0 instrument and are uncorrected. Fluorescence spectra were recorded on a Photon Technology International fluorimeter with a 10 mm cuvette. The excitation and emission wavelength band paths were both set at 10 nm. Solutions of each analyte (PPi, Pi, ATP, ADP, and others) were prepared by dissolving the sodium salt of the anionic species in deionized water. Stock solutions of **1**·Zn(II), **1**·Cu(II) and **6** were prepared by dissolving them separately in deionized water (each 1.0 mM).

6-Bromo-2-methoxynaphthalene-1-carbaldehyde (2)

A solution of 6-bromo-2-methoxynaphthalene (1.19 g, 5 mmol) in anhydrous CH_2Cl_2 (10 mL) was added to a solution of $TiCl_4$ (2.02 g, 10.9 mmol) and α,α-dichloromethylmethyl ether (642 μL, 5.6 mmol) in anhydrous CH_2Cl_2 (8 mL) at 0 °C. The resulting solution was stirred for 1 h at the same temperature, and then it was raised to room temperature and further stirred for 10 h before quenching with dilute aqueous HCl. The reaction mixture was subjected to an extractive workup with dichloromethane, the

organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on silica gel (hexane/EtOAc = 2/1) to afford **2** as a yellow solid (1.07 g, 80%). m.p: 96–98 °C; IR (KBr, cm^{-1}) 1664; ^1H NMR ($CDCl_3$, 300 MHz, 298 K) δ 10.85 (1H, d, J = 3.3 Hz), 9.18 (1H, dd, J = 9.3, 2.7 Hz), 7.98–7.91 (2H, m), 7.66 (1H, ddd, J = 9.3, 4.8, 2.2 Hz), 7.33 (1H, dd, J = 9.2, 4.6 Hz), 4.06 (3H, s); ^{13}C NMR ($CDCl_3$, 75 MHz, 298 K) δ 191.8, 164.1, 136.66, 133.1, 130.2, 130.1, 129.9, 127.1, 118.7, 116.8, 113.9, 56.8; MS (ESI): m/z 265.8.

5-Formyl-6-methoxynaphthalene-2-carbonitrile (3)

To a solution of compound **2** (532 mg, 2 mmol) in anhydrous NMP (15 mL) was added copper(I) cyanide (269 mg, 3 mmol) under argon. The mixture was heated at 135 °C for 8 h. After being cooled to room temperature, the reaction mixture was poured into an aqueous NH_4Cl solution (25 mL), and it was stirred at room temperature for 1 h. The mixture was then partitioned between EtOAc and water. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent under reduced pressure, and the residue was purified by flash column chromatography on silica gel (hexane/EtOAc = 1/1) to afford the desired compound **3** as a yellow solid (253 mg, 60%). mp: 234–238 °C; IR (KBr, cm^{-1}): 2229, 1659; ^1H NMR (DMSO-d_6, 300 MHz, 298 K) δ 10.73 (1H, s), 9.18 (1H, d, J = 8.9 Hz), 8.58 (1H, s), 8.41 (1H, d, J = 9.2 Hz), 7.91 (1H, d, J = 8.9 Hz), 7.78 (1H, d, J = 9.2 Hz), 4.11 (3H, s); ^{13}C NMR (DMSO-d_6, 75 MHz, 298 K) δ 191, 165.8, 138.4, 134.7, 132.5, 130.1, 126.9, 125.1, 118.9, 115.8, 115.2, 106.8, 57.2; HRMS FAB-(+): m/z calcd for $C_{13}H_9O_2N$ (M^+): 211.0633, found 211.0634.

5-Formyl-6-hydroxynaphthalene-2-carbonitrile (4)

To a solution of compound **3** (422 mg, 2 mmol) in anhydrous CH_2Cl_2 (20 mL) was added dropwise 1.0 M BBr_3 in CH_2Cl_2 (3 mL, 3 mmol) at 0 °C under argon. The resulting mixture was stirred at 0 °C for 1 h, and then the temperature was raised to room temperature and it was further stirred for 8 h. To the mixture at 0 °C was added a 5% aqueous $NaHCO_3$ solution (10 mL), and then the mixture was warmed to room temperature with stirring for 30 min and then partitioned between CH_2Cl_2 and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (hexane/CH_2Cl_2 = 1/9) to afford **4** as a yellow solid (217 mg, 55%). mp: 217–220 °C; IR (KBr, cm^{-1}): 2225, 1630; ^1H NMR (CD_2Cl_2, 300 MHz, 298 K) δ 13.34 (1H, d, J = 3.3 Hz), 10.8 (1H, d, J = 3.2 Hz), 8.46 (1H, d, J = 8.8 Hz), 8.2 (1H, s), 8.06 (1H, d, J = 9.2 Hz), 7.79 (1H, dd, J = 8.8, 1.5 Hz), 7.28 (1H, d, J = 9.2 Hz); ^{13}C NMR (CD_2Cl_2, 75 MHz, 298 K) δ 193.8, 167.3, 139.4, 135.5, 135.4, 130.6, 127.4, 121.8, 120.6, 119.1, 111.8, 108.7; HRMS FAB-(+): m/z calcd for $C_{12}H_8O_2N$ (M^+): 197.0477, found 197.0476.

5-{[Bis(pyridin-2-ylmethyl)amino]methyl}-6-hydroxy-naphthalene-2-carbonitrile (1)

Bis(2-pyridylmethyl)amine (144 μL, 0.8 mmol) was added dropwise to a solution of **4** (197 mg, 1 mmol) in anhydrous CH_2Cl_2 (10 mL). The mixture was stirred for 2 h before $NaBH(OAc)_3$ (699 mg, 3.3 mmol) was added, and the resulting mixture was further stirred for 8 h. The reaction mixture was diluted with dichloromethane (100 mL), partitioned in 2% aqueous $NaHCO_3$ solution (10 mL) and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH = 19/1) to afford **1** as a white solid (153 mg, 40%). mp: 154–158 °C; IR (KBr, cm^{-1}): 2223; ^1H NMR ($CDCl_3$, 300 MHz, 298 K) δ 8.62–8.59 (2H, m), 8.11 (1H, d, J = 1.5 Hz), 7.98 (1H, d, J = 8.9 Hz), 7.74 (1H, d, J = 8.9 Hz), 7.67 (2H, doublet of triplet, J = 5.9, 1.8 Hz), 7.54 (1H, dd, J = 8.8, 1.7 Hz), 7.35 (1H, s), 7.32 (2H, d, J = 3.2 Hz), 7.28 (1H, s), 7.24–7.2 (2H, m), 4.26 (2H, s), 3.91 (4H, s); ^{13}C NMR ($CDCl_3$, 75 MHz, 298 K) δ 159.2, 157.7, 149.0, 137.2, 135.8, 134.8, 130.3, 127.6, 127.1, 123.8, 123.3, 122.7, 121.9, 120, 114.3, 105.6, 58.9, 50.3; HRMS FAB-(+): m/z calcd for $C_{24}H_{21}ON_4$ (M^+): 381.1715, found. 381.1716.

5-[(Bis-pyridin-2-ylmethyl-amino)-methyl]-6-methoxy-naphthalene-2-carbonitrile (5)

Bis-(2-pyridylmethyl)amine (144 μL, 0.8 mmol) was added dropwise to a solution of **3** (211 mg, 1 mmol) in anhydrous CH_2Cl_2 (10 mL). The mixture was stirred for 2 h before treatment with $NaBH(OAc)_3$ (466 mg, 2.2 mmol), which was further stirred for 8 h. The reaction mixture was diluted with dichloromethane (80 mL), partitioned with 2% aqueous $NaHCO_3$ solution (8 mL) and water. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel (CH_2Cl_2/MeOH = 20/1) to afford **8** as a yellow solid (217 mg, 55%). mp: 175–178 °C; IR (KBr, cm^{-1}): 2222; ^1H NMR ($CDCl_3$, 300 MHz, 298 K) δ 8.52–8.49 (2H, m), 8.09–8.06 (2H, m), 7.75 (1H, d, J = 9 Hz), 7.57 (2H, doublet of triplet, J = 7.8, 1.8 Hz), 7.48 (1H, dd, J = 9, 1.8 Hz), 7.33–7.26 (3H, m), 7.13 (1H, dd, J = 5.1, 1.2 Hz), 7.1 (1H, dd, J = 5.1, 1.2 Hz), 4.15 (2H, s), 3.95 (3H, s), 3.83 (4H, s); ^{13}C NMR ($CDCl_3$, 75 MHz, 298 K) δ 160, 158.3, 149, 136.3, 135.9, 134.4, 130.3, 127.9, 126.5, 126.3, 123.5, 122.1, 120, 119.8, 114.4, 106.6, 61, 56.5, 48.4; HRMS FAB-(+): m/z calcd for $C_{25}H_{22}ON_4$ (M^+): 394.4684, found. 394.4681.

1·Zn(II)

To a solution of DPA **1** (57.2 mg, 0.15 mmol) in MeOH (5 mL) was added $Zn(NO_3)_2{\cdot}6H_2O$ (44.6 mg, 0.15 mmol), and the resulting mixture was stirred overnight. Solvent was removed under vacuum and the obtained sticky solid was recrystallized twice from MeOH/CH_2Cl_2 (9/1) to give the complex **1**·Zn(II) as white solid (54 mg, 81%). ^1H NMR (CD_3OD, 300 MHz, 298 K) δ 8.63 (2H, d, J = 4.5 Hz), 8.24 (1H, d, J = 9 Hz), 8.08 (1H, d, J = 1.5 Hz), 7.80 (2H, dt, J = 7.8, 1.8 Hz), 7.65 (1H, dd, J = 9, 1.8 Hz), 7.55 (1H, d, J = 9 Hz), 7.48–7.43 (2H, m), 7.12 (1H, d, J = 7.8 Hz), 6.87 (1H, d, J = 1.5 Hz), 4.34, 4.16 (2 × 2H, AB type, J = 16.2 Hz), 4.23 (2H, s); ^{13}C NMR (CD_3OD, 75 MHz, 298 K) δ 156.7, 149.4, 142.2, 137, 136.1, 133, 129.2, 129, 126.3, 125.1, 124.4, 121.2, 120.2, 115.4, 107.6, 60.2, 51; HRMS FAB-(+): m/z calcd for $C_{24}H_{19}ON_4Zn$ (M^+): 443.0850, found 443.0853.

1·Cu(II)

This copper(II) complex was prepared similarly as above by addition of $Cu(NO_3)_2{\cdot}2.5H_2O$ to a solution of DPA **1** in MeOH at

room temperature. It was purified by recrystallization twice from MeOH/CH_2Cl_2 (9/1) to give **1**·Cu(II) in 85% yield. HRMS FAB-(+): *m/z* calcd for $C_{24}H_{20}O_4N_5Cu$ (M + NO_3^-): 505.0811, found. 505.0806.

Zn(II) complex 6

This zinc(II) complex was prepared similarly as above by addition of $Zn(ClO_4)_2 \cdot 6H_2O$ to a solution of compound **5** in MeOH at room temperature. ^{1}H NMR (CD_3OD, 300 MHz, 298 K) δ 8.63 (2H, d, J = 4.5 Hz), 8.36–8.31 (1H, m), 8.19 (1H, s), 7.97–7.92 (2H, m), 7.76 (1H, dd, J = 9, 1.8 Hz), 7.63–7.59 (2H. m), 7.28 (2H, d, J = 7.2 Hz), 7 (1H, d, J = 8.7 Hz), 4.51, 4.27 (2 × 2H, AB, J = 16.2 Hz), 4.34 (2H, s), 3.35 (3H, s).

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