Pyrophosphate-induced reorganization of a reporter–receptor assembly *via* boronate esterification; a new strategy for the turn-on fluorescent detection of multi-phosphates in aqueous solution†

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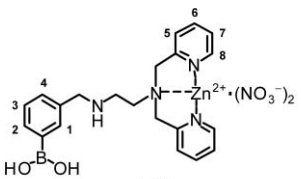
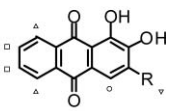
A new strategy for the fluorescent detection of multi-phosphates in aqueous solution is presented here. Zn^{II}–DPA(DPA = dipicolylamine)-appended phenylboronic acid **1·Zn** forms an assembly with alizarin dye in MeOH–10 mM HEPES (1 : 1 v/v) containing 10 mM NaCl at pH 7.4 at 25 °C, in which the dye binds favorably to the coordinated zinc(II) in the Zn^{II}–DPA moiety. Addition of pyrophosphate (PPi) as a putative analyte causes reorganization of the complex to produce an alternative boronate ester assembly, which causes an increase in fluorescence, detectable by the naked eye. It is interesting to note that the system exhibited PPi-selectivity over other phosphates such as ATP (adenosine 5′-triphosphate), ADP (adenosine 5′-diphosphate), AMP (adenosine 5′-monophosphate) and Pi (inorganic phosphate); the competitive assay employed to determine the apparent association constants of **1·Zn** with the anion analytes allows us to estimate that the binding with PPi [$(1.6 \pm 0.04) \times 10^6$ M^{-1}], is 10-fold and 84-fold higher than with ATP and ADP, respectively. The sensing mechanism of **1·Zn** in the presence of alizarin dye is explored using pH titrations and structural information is obtained using NMR.

Introduction

Anions are ubiquitous in biological systems[1] that play significant roles in the wide areas of biology, pharmacy, and environmental science. The design of receptor systems for anion recognition has therefore developed into a key area of supramolecular chemistry,[2] whereby, in particular, fluorescent chemosensors have received considerable attention due to their analytical applications.[3] One route to prepare molecular sensor systems, avoiding extensive synthetic chemistry, is through the development of self-organized receptor–reporter systems, obtained by linking molecular units through reversible interactions.[4] For instance, the indicator displacement assay, pioneered by Anslyn and Nguyen, is a valuable means of analyte detection.[5] The study presented here has been driven by a new approach that involves an anion-induced reorganization of the reporter–receptor assembly. The idea is that the binding of an anion will induce a structural change in the system, followed by a switch in the optical properties of the reporter. Our system incorporates a catechol-containing dye such as alizarin (reporter) and Zn^{II}–DPA(DPA = dipicolylamine)-appended phenylboronic acid **1·Zn**, which serves as a receptor. The role of the phenylboronic acid segment is significant; it can form a complex with the catechol-containing dye *via* boronate esterification and induce a change in the optical properties.[6] As part of this investigation we discovered that pyrophosphate (PPi), which binds strongly to the Zn^{II}–DPA, causes the reporter dye to be expelled from the Zn^{II}–DPA, then the reporter forms a boronate ester with the boronic acid segment of **1·Zn**. This change in the assembly mode causes the fluorescence intensity to increase. The phenomenon allows us to design a new type of chemosensor for multi-phosphates. Such detection of multi-phosphates is worthy of investigation because there is a demand for their analytical detection in clinical applications.[7] In this regard, while fluorogenic receptors capable of sensing PPi in aqueous media are fascinating targets, their exploration is still in its infancy, presumably due to the fact that it is not easy to detect PPi selectively in the presence of other kinds of phosphate such as ATP (adenosine 5′-triphosphate) and ADP (adenosine 5′-diphosphate) in aqueous solution.[8]



1·Zn



R = SO_3Na: ARS
R = H: alizarin

Results and discussions

Synthesis

We designed phenylboronic acid derivative **1·Zn** as the receptor moiety in our system; it is well-known that Zn^{II}–DPA serves as a suitable phosphate-binding site in aqueous solution for developing not only artificial enzymes[9] but also chemosensors.[10]

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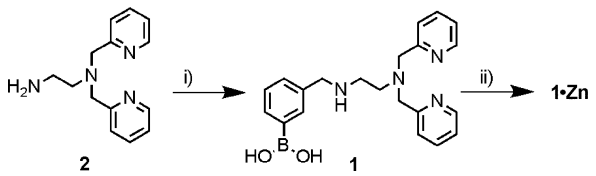
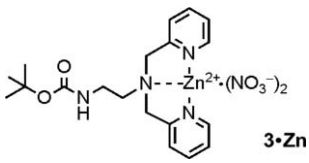
† Electronic supplementary information (ESI) available: Fluorescence spectra of ARS plus **1·Zn** upon adding PPi; fluorescence spectra of ARS plus phenylboronic acid upon adding PPi; $^1H,^1H$ COSY spectrum of alizarin plus **1·Zn** with PPi; fluorescence spectra of ARS upon adding incremental amounts of **3·Zn**. See DOI: 10.1039/b808027e

The synthesis is straightforward as shown in Scheme 1. The reductive amination of *N*,*N*-bis(2-pyridylmethyl)ethylenediaime **2**[11] with 3-formylphenylboronic acid was carried out using $NaBH_4$, and the resulting DPA-appended phenylboronic acid **1** was allowed to react with $Zn(NO_3)_2$ to yield the target **1**·**Zn**. The control **3**·**Zn** was also synthesized from the Boc-protected compound, *N*-*tert*-butoxycarbonyl-*N*′,*N*′-bis(2-pyridylmethyl)ethylenediamine, **3**[11] in a similar manner. The compounds were characterized using spectroscopic methods, which are found in the Experimental section. The ^{1}H NMR spectrum of the new compound was insightful as the methylene resonances (3.87 ppm, s) of the DPA part of **1** were altered to AB double doublets (4.25 ppm, d, J = 17.1 Hz; 4.38 ppm, d, J = 17.0 Hz) upon metallation with Zn^{II}, for example.



Scheme 1 Synthesis of **1**·**Zn**. *Reagents and conditions:* (i) 3-formylphenylboronic acid then $NaBH_4$, EtOH, 12%; (ii) $Zn(NO_3)_2{\cdot}6H_2O$, MeOH, 89%.



Evaluation

Initially, to get an insight into the fluorescence behavior of ARS (alizarin red S) as the reporter, stepwise titrations using the Zn^{II}-free ligand **1** were carried out in MeOH–10 mM HEPES (1 : 1 v/v) containing 10 mM NaCl at pH 7.4 at 25 °C (Fig. 1) (HEPES = 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid). Although ARS has almost no emission under these conditions, the fluorescence intensity ranging from 550 nm to 600 nm increased upon the addition of incremental amounts of **1** (Fig. 1(a)). This phenomenon can be explained on the basis of boronate esterification between ARS and **1**,[6] the binding constant being estimated as $(3.8 \pm 0.44) \times 10^4$ M^{-1}. One can compute from the binding constant that 91% of ARS can be converted to the ARS–**1** assembly in the presence of 5 equiv. of **1** under these conditions. Next, the addition of Zn^{II} to the solution involving ARS (50 μM) and **1** (5 equiv.) under similar conditions induced a "turn-off" in the fluorescence spectra (Fig. 1(b)). The quenched spectrum with 5.3 equiv. of Zn^{II} is almost consistent with that of ARS in the presence of 5 equiv. of **1**·**Zn**, suggesting that coordinated Zn^{II} in the Zn^{II}–DPA species interacts with the ARS. Evidence for the assembly formation came from ESI-MS spectroscopic data using alizarin and **1**·**Zn** in MeOH (m/z = 691.7635 (calcd for $[C_{36}H_{34}BN_4O_6Zn]^+$; 692.1894). The fluorescence properties of ARS under several conditions in MeOH–H_2O (1 : 1 v/v) were also investigated by carrying out spectrofluorometry at varying pH where a solution containing excess acid was titrated with standard base (Fig. 2). ARS has almost no fluorescence over a large pH range from 2 to 12 (Fig. 2; ●), whereas in the presence of **1**·**Zn** (5 equiv.) the fluorescence intensity at 586 nm increased up to pH 6, followed by a decrease in the fluorescence intensity (Fig. 2; ■). We can ascribe the observed fluorescence enhancement to the formation of an ARS–**1**·**Zn** ensemble *via* boronate esterification. In contrast, the decrease in the fluorescence intensity at pH > 6 is due to the more favorable interaction between the catechol segment of ARS and the coordinated Zn^{II} in the Zn^{II}-DPA species. The change in the assembly mode is probably controlled by the p*K*a value of ARS which is 5.5.[12] On another front, the presence of PPi as a putative analyte in the solution involving ARS and **1**·**Zn** produced a different pH profile when compared to the PPi-free solution (Fig. 2; ▲); the fluorescence intensity increases more effectively and reaches a maximum between pH 6 to 8. This significant fluorescence enhancement under neutral conditions motivated us to set up the conditions for applying the ARS–**1**·**Zn** assembly to PPi sensing. The fluorescence titration of ARS (50 μM) with PPi in the presence of **1**·**Zn** (5 equiv.) was carried out in MeOH–10 mM HEPES (1 : 1 v/v) containing 10 mM NaCl at pH 7.4 at 25 °C; as expected, the fluorescence intensity at 586 nm increases upon addition of PPi, with factors of 2.75 (250 μM of PPi) (see ESI†), the behavior being detected by the naked-eye. The presence of the Zn^{II}–DPA segment is significant for the response. Indeed, the use of Zn^{II}–DPA-free phenylboronic acid instead of **1**·**Zn** induced almost no fluorescence enhancement by adding PPi under similar conditions (ESI†).

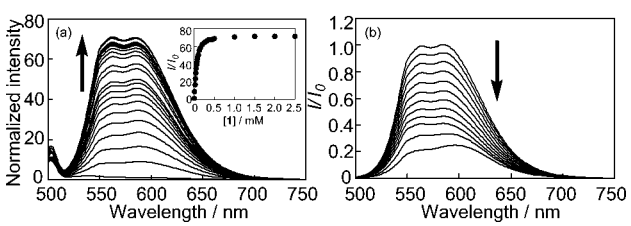


Fig. 1 (a) Change in the fluorescence spectra of ARS (50 μM) upon adding **1** in MeOH–10 mM HEPES (1 : 1 v/v) containing 10 mM NaCl at pH 7.4 at 25 °C, λ_{ex} = 501 nm; (b) Change in the fluorescence spectra of ARS (50 μM) in the presence of **1** (250 μM) upon adding Zn^{II} in MeOH–10 mM HEPES (1 : 1 v/v) containing 10 mM NaCl at pH 7.4 at 25 °C, λ_{ex} = 480 nm. I_0 is fluorescence intensity under the Zn^{II}-free conditions.

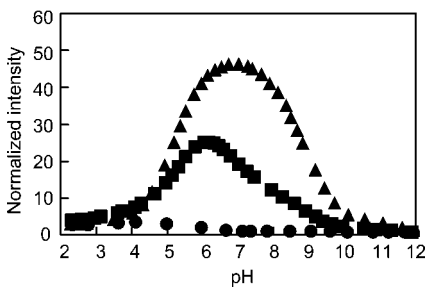


Fig. 2 Spectrofluorimetric pH-titrations of ARS (●), ARS plus **1**·**Zn** (■) and ARS plus **1**·**Zn** with PPi (▲) in MeOH–H_2O (1 : 1 v/v) containing 10 mM NaCl at 25 °C; [ARS] = 50 μM, [**1**·**Zn**] = 250 μM, [PP_i] = 250 μM, λ_{ex} = 480 nm; λ_{em} = 586 nm.

The binding profile was investigated based on ^{1}H NMR data whereby alizarin was employed in place of ARS because the H_3 signal (▽) of alizarin is considered to be diagnostic for boronate ester formation;[6c] Fig. 3 shows how the aromatic protons of each

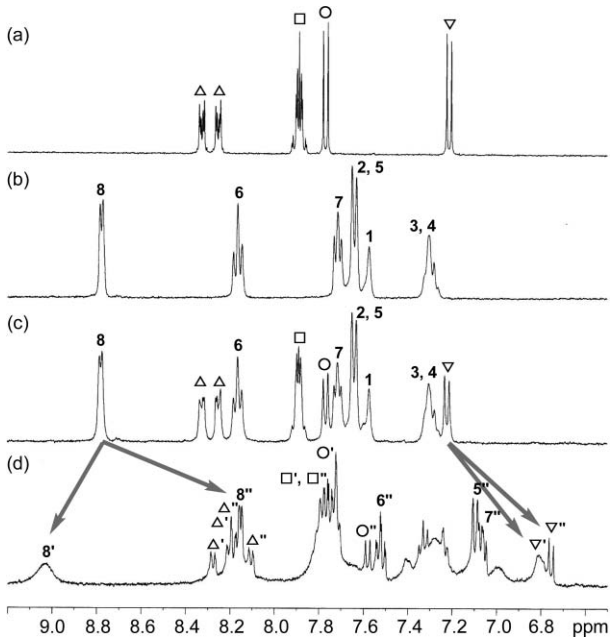
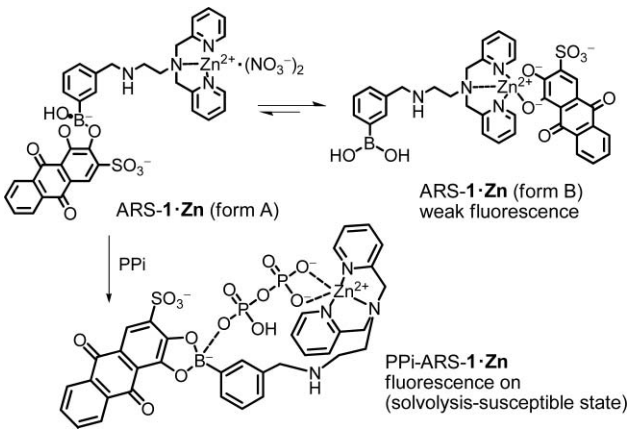


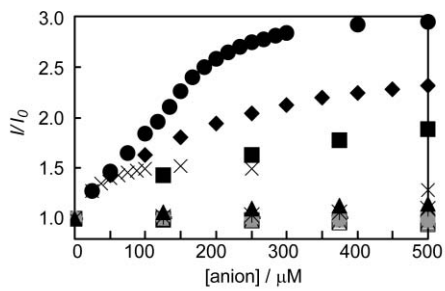
Fig. 3 ^{1}H NMR spectra in CD_3OD–D_2O (9 : 1 v/v) (400 MHz) at 23 °C. [alizarin] = 2.4 mM, [**1**·**Zn**] = 2.4 mM; (a) alizarin, (b) **1**·**Zn**, (c) alizarin plus **1**·**Zn**, (d) alizarin plus **1**·**Zn** with PPi. The spectrum of (d) was obtained after (solid (PP_i)–liquid (2.4 mM of alizarin and **1**·**Zn** in CD_3OD–D_2O (9 : 1 v/v))) two-phase extraction in view of the low solubility of PP_i under NMR detectable conditions.

component reflect the PPi-induced organization in the assembly. No perturbation of the chemical shifts for alizarin and **1**·**Zn** was observed when they are mixed (Fig. 3(a) and (b) *vs.* Fig. 3(c)). However, when PPi was added to the mixture the chemical shifts changed dramatically and became complicated (Fig. 3(d)): an additional ^{1}H,^{1}H COSY measurement (ESI†) helped us to assign the signals; the resonances arising from alizarin were clearly distinguishable signals in the presence of PPi. These observations can be explained on the basis of boronate ester formation whereby the PO–B (sp^3) dative bond would be partially subject to solvolysis in protic media.[13] The entirely up-field shift of the resonances here fully supports the formation of a boronate ester;[6c] for example, the H_∇ signal was significantly shifted from 7.22 (d, $J = 8.3$ Hz) to 6.75 (d, $J = 8.0$ Hz) and 6.81 ppm (brd). In contrast, we notice that the α-protons of the pyridine ring (H_8) were down-field shifted from 8.78 (d, $J = 4.6$ Hz) to 9.02 ppm and somewhat broadened upon addition of PPi, indicating that PPi binds to the coordinated Zn^{II} in the Zn^{II}-DPA species.[8c] These insights allow us to postulate that the PPi is ditopically bound within the alizarin–**1**·**Zn** assembly, both to the Zn^{II} and the boronate ester segment. In ^{11}B NMR measurements, an upfield shift ($\Delta\delta = 15.82$ ppm) of the boron signal was observed upon the addition of PPi in a MeOH–D_2O (9 : 1 v/v) solution of **1**·**Zn** (2.4 mM) and alizarin (2.4 mM). However, we also detected in the spectra signals assignable to Zn^{II}-free DPA; 8.15 (d, $J = 4.8$ Hz, $H_{8''}$), 7.52 (td, $J = 7.7$, 1.7 Hz, $H_{6''}$), 7.10 (d, $J = 7.9$ Hz, $H_{5''}$) and 7.06 ppm (dd, $J = 7.5$, 5.1 Hz, $H_{7''}$). The singlet peak at 3.77 ppm is also assignable to the Zn^{II}-free NCH_2Pyr. One can envisage that the PPi-associated alizarin–**1**·**Zn** assembly is susceptible to solvolysis to afford solvent-inserted alizarin-**1** and $Zn_2P_2O_7$. Buffer-free conditions during the NMR measurements may facilitate decomposition of the Zn^{II}–DPA segment. Taken together, a plausible mechanism for the PPi-induced fluorescence enhancement in the assembly is illustrated in Scheme 2. ARS binds to **1**·**Zn** efficiently to form the ARS–**1**·**Zn** assembly, which is equilibrating between the boronate ester form (A) and the catechol–Zn^{II} form (B). Under neutral conditions, form (B) is more favoured than form (A) and suppresses the fluorescence arising from ARS. When PPi is added into the solution, PPi favorably binds to the (A) form, producing a shift in the equilibrium towards (A). The ditopic binding of PPi to (A) affords the ternary complex, PPi–ARS–**1**·**Zn**, accompanied by a fluorescence enhancement.



Scheme 2 A plausible mechanism for the PPi-induced fluorescence enhancement.

The anion selectivity of the ARS–**1**·**Zn** system was then examined using PPi, ATP, ADP, AMP (adenosine 5′-monophosphate), Pi (HPO_4^{2-} mainly equilibrates with $H_2PO_4^-$ under the neutral conditions) and other biologically as well as chemically important anions, in MeOH–10 mM HEPES (1 : 1 v/v) containing 10 mM NaCl at pH 7.4 at 25 °C. As shown in Fig. 4, when titrations with PPi were performed a significant enhancement in the fluorescence intensity was obtained. Almost similar but less effective responses were obtained in the case of ATP and ADP addition, whereas AMP as well as Pi induced no response in the fluorescence spectra. Consequently, ARS–**1**·**Zn** shows a selective response towards phosphates in the following order; PPi > ATP > ADP > AMP > Pi. The addition of carboxylates as putative oxoanions other than phosphates induced either no (for AcO^-) or a low response (for



PPi (●), ATP (◆), ADP (■), AMP (▲), Pi (∗), citrate (×), AcO^- (–), F^- (○), Br^- (◇), I^- (□), HCO_3^- (△), NO_3^- (●), SO_4^{2-} (◆), ClO_4^- (■), N_3^- (▲).
The measurements were carried out in MeOH–10 mM HEPES (1:1 v/v) containing 10 mM NaCl at pH 7.4 at 25 °C, λ_{ex} = 480 nm.

Fig. 4 Plots of fluorescence intensity at 586 nm of ARS (50 μM) and 5 equiv. of **1**·**Zn** as a function of anion concentration.

citrate). Also one can detect no fluorescence enhancement under these conditions using monovalent (HCO_3^-, F^-, Br^-, I^-, NO_3^-, ClO_4^- and N_3^-) as well as divalent (SO_4^{2-}) anions. Subsequently we tried to determine the association constants from the titration data. However, the titration curve obtained for the addition of PPi (0–500 μM) displayed a somewhat sigmoidal progression because of multiple equilibria as well as an excess amount of **1**·**Zn** (also capable of binding to the anion) making it impossible for simple nonlinear curve fitting to reproduce the observed progression. Thus, although the anion-triggered fluorescence enhancement is not due to the regular indicator displacement assay, we decided to determine the apparent binding constant of PPi to the receptor **1**·**Zn** (K_a) in line with the assumption based on the equilibrium from eqn (1)

$$\text{ARS–}\mathbf{1}\cdot\mathbf{Zn} + \text{G} \rightleftarrows \text{G–}\mathbf{1}\cdot\mathbf{Zn} + \text{ARS}^* \quad (1)$$

where ARS–**1**·**Zn** is regarded as form B in Scheme 2, G is the guest anion, and G–**1**·**Zn** and ARS* represent a G-coordinated Zn^{II}–DPA entity and a boronate esterified ARS, respectively. The mass balance equation and the equilibrium constants (K_1 and K_a) were used to define P and Q.[14] For this approach, the binding constant of ARS to Zn^{II}–DPA in the form B could be estimated as K_1; we carried out the fluorescent titration of ARS upon adding incremental amounts of phenylboronic acid-free Zn^{II}-DPA, **3**·**Zn**, being estimated for K_1 as 5.4×10^4 M^{-1} (ESI†).

$P = [\mathbf{1}\cdot\mathbf{Zn}]_t - 1/(QK_1) - [\text{ARS}^*]_t/(Q + 1)$

$Q = [\text{ARS}^*]/[\text{ARS} - \mathbf{1}\cdot\mathbf{Zn}]$

$Q = (I - I_0)/(I_{lim} - I)$

Q is termed the indicator ratio, and can be obtained using the fluorescence intensity of ARS when coordinated to Zn^{II} in the form B (I_0) and when existing as the esterified boronate (I_{lim}). Fig. 5 shows the relationship between G_t/P *vs.* Q, where in the case of PPi it is hard to obtain a linear relationship between them. In particular, at $Q < 2.5$ ($[PPi]_t = 167$ μM, 3.34 equiv.), the deviation became large. This result could be explained on the basis of an excess amount of **1**·**Zn** compared to that of ARS in the solution; PPi could bind more efficiently to free **1**·**Zn** than to ARS-bound **1**·**Zn**. The linearity was consequently obtained ranging from 167 to 300 μM of $[PPi]_t$, which allows us to elucidate the apparent association constant K_a by dividing K_1 by the slope of the plot (Fig. 5). Three individual measurements afforded $(1.6 \pm 0.04) \times 10^6$ M^{-1} as the K_a value with PPi. Similar analyses were carried out for other phosphates (ATP and ADP), as shown in Fig. 5, to elucidate the K_a with ATP and ADP as $(1.6 \pm 0.28) \times 10^5$ M^{-1} and $(1.9 \pm 0.19) \times 10^4$ M^{-1}, respectively. It is noteworthy that **1**·**Zn** exhibited selective binding to PPi by a factor of 10 and 84, compared to ATP and ADP, respectively. This selectivity is presumably due to the total anionic density of the P–O involved in the interaction between ARS-**1**·**Zn** and multi-phosphorylated species.[8e]

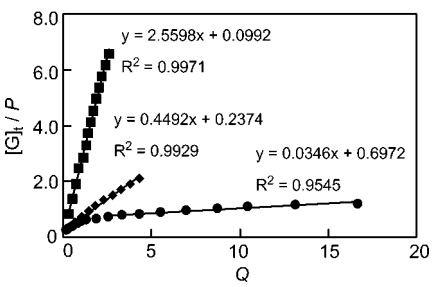


Fig. 5 A competitive titration algorithm to determine the association constants of **1**·**Zn** with the multi-phosphates; PPi (●), ATP (♦), ADP (■).

Conclusions

We synthesized the Zn^{II}–DPA-appended phenylboronic acid **1**·**Zn**, which interacts with ARS in aqueous solution to form an ARS–**1**·**Zn** assembly with a low fluorescence intensity under neutral conditions. The binding of PPi could induce a structural variation in the assembly, followed by a switch in its optical properties. Although the preliminary results represented here require an improvement in the responsive ability toward target anions, our proposed system has conceptual novelty with regard to the sensing principle for the detection of multi-phosphates in aqueous media. One merit of the system is that a judicious choice of catechol-containing dye as the indicator, as well as a change in the metal ion coordinated to the DPA, would allow us to tune not only spectral but also selectivity features as required. Also, given the rich chemistry of boronic acids, variation of the receptor moiety is possible. Further elaborated systems based on this concept are now underway.

Experimental

NMR spectra were taken on Bruker DRX-400 or DPX-400 (^{1}H: 400 MHz; ^{13}C: 100.7 MHz; ^{11}B: 128 MHz) spectrometers. Chemical shifts (δ) are reported downfield from the initial standard Me_4Si. For ^{11}B NMR 15% BF_3–Et_2O was employed as the external standard. Electrospray ionization mass spectra were obtained on a Mariner System 5231 spectrometer whereas a Jeffermine D2000 was employed for the calibration. Fast atom bombardment (FAB) mass spectra were obtained on a JEOL JMS-DX 303 double focusing spectrometer where *m*-nitrobenzyl alcohol was used as a matrix. Elemental analyses were obtained on an EISON EA1108 or ThermoFinigan Flash EA1112. Fluorescence and absorption spectra were measured using a JASCO FP-6300 and Shimadzu UV-3100PC spectrophotometer, respectively. Reagents used for the synthesis were commercially available and used as supplied. ARS and alizarin were recrystallized from EtOH. Dry EtOH was prepared according to standard procedures. The MeOH and H_2O for spectroscopic measurements were purchased as analytical grade and used as received.

N-3-Dihydroxyborylbenzyl-*N*′,*N*′-bis(2-pyridylmethyl) ethylenediamine 1

Under an Ar atmosphere, *N*,*N*-bis(2-pyridylmethyl)ethylenediamine (4.60 g, 19.0 mmol) and 3-formylphenylboronic acid (2.85 g, 19.0 mmol) were dissolved in dry EtOH (300 mL) that had been degassed by three freeze–pump–thaw cycles. The solution, containing molecular sieves 3 Å (10 g), was stirred overnight at room temperature. After checking the progress of the reaction by TLC, a dry EtOH solution (80 mL) of $NaBH_4$ (1.44 g, 38.1 mmol) was added to the solution and the mixture was further stirred at room temperature for 1 h. After filtration the solution was evaporated *in vacuo*. The residue was partitioned between AcOEt (300 mL) and H_2O (300 mL) and the water phase was extracted

with CH_2Cl_2 (50 mL × 10). The organic phase was dried with dry Na_2SO_4 and filtered. The material obtained was chromatographed on silica gel (Wakogel C-300) using a gradient of MeOH (0–100% (v/v)) in CH_2Cl_2 as an eluent, and then washed with AcOEt and Et_2O. In this way, 843.9 mg of **1** was obtained in 12% yield.

^{1}H NMR (400 MHz, CD_3OD) δ 8.25 (d, J = 4.5 Hz, 2H), 7.64 (td, J = 7.7, 1.7 Hz, 2H), 7.62 (d, J = 7.1 Hz, 1H), 7.58 (s, 1H), 7.28 (t, J = 7.4 Hz, 1H), 7.17–7.23 (m, 5H), 4.11 (s, 2H), 3.87 (s, 4H), 3.25 (t, J = 5.4 Hz, 2H), 3.04 (t, J = 5.5 Hz, 2H); ^{13}C NMR (100.7 MHz, 50 mM in CD_3OD) δ 159.9, 149.8, 138.6, 135.3, 135.1, 131.4, 128.3, 127.2, 124.9, 123.8, 60.6, 53.0, 52.7, 46.4; ESI MS: m/z 377 ([M + H]$^+$); elemental analysis: anal. calcd for $C_{21}H_{25}BN_4O_2 \cdot 0.3\ H_2O$: C 66.09; H 6.76; N 14.68%, found: C 65.96; H 6.64; N 14.28%.

N-3-Dihydroxyborylbenzyl-*N*′,*N*′-bis(2-pyridylmethyl) ethylenediamine zinc (II) complex 1·Zn

Ligand **1** (514.4 mg, 1.37 mmol) and $Zn(NO_3)_2 \cdot 6H_2O$ (407.6 mg, 1.37 mmol) were dissolved in MeOH (45 mL). The resulting mixture was stirred for 1 h at room temperature. After removal of the solvent *in vacuo* the resulting residue was recrystallized with THF, washed with Et_2O, and dried under heat at 40 °C. In this way, 687.9 mg of **1·Zn** was obtained in 89% yield.

^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (d, J = 4.6 Hz, 2H), 8.13 (app. t, J = 7.7 Hz, 2H), 7.66 (t, J = 6.4 Hz, 2H), 7.61 (d, J = 7.9 Hz, 2H), 7.54 (s, 1H), 7.50 (d, J = 6.7 Hz, 1H), 7.33 (d, J = 7.4 Hz, 1H), 7.29–7.22 (m, 1H), 4.38 (d, J = 17.0 Hz, 2H), 4.25 (d, J = 17.1 Hz, 2H), 4.11 (brs, 1H), 3.49 (brs, 1H), 2.94 (brs, 2H), 2.63 (s, 2H); ^{13}C NMR (100.7 MHz, CD_3OD) δ 157.1, 149.7, 142.6, 136.3, 136.0, 135.6, 134.9, 134.4, 132.4, 131.8, 129.1, 126.2, 125.7, 59.1, 54.2, 53.4, 44.6; ESI MS: m/z 219 ([M − 2(NO$_3$)]$^{2+}$; elemental analysis: anal. calcd for $C_{21}H_{25}BN_6O_8Zn \cdot 0.5\ H_2O \cdot 0.5$ MeOH: C 42.70; H 4.78; N 14.23%, found: C 42.50; H 4.42; N 14.11%.

N-*tert*-Butoxycarbonyl-*N*′,*N*′-bis(2-pyridylmethyl) ethylenediamine zinc (II) complex 3·Zn

N-*tert*-Butoxycarbonyl-*N*′,*N*′-bis(2-pyridylmethyl)ethylenediamine (500 mg, 1.46 mmol) and $Zn(NO_3)_2 \cdot 6H_2O$ (297.5 mg, 1.46 mmol) were dissolved in MeOH (50 mL). The resulting mixture was stirred for 1 h at room temperature. After removal of the solvent *in vacuo* the resulting residue was recrystallized with MeOH. In this way, 6.26 g of **3·Zn** was obtained in 44% yield.

^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (d, J = 4.8 Hz, 2H), 8.19 (td, J = 7.7, 1.6 Hz, 2H), 7.68–7.73 (m, 4H), 4.51 (d, J = 16.2 Hz, 2H), 4.19 (d, J = 16.2 Hz, 2H), (t, J = 6.7 Hz, 2H), 2.76 (t, J = 6.6 Hz, 2H), 1.38 (s, 9H); ^{13}C NMR (100.7 MHz, CD_3OD) δ 158.52, 156.42, 149.42, 142.94, 126.58, 126.36, 81.05, 58.11, 53.99, 36.60, 28.67; ESI MS: m/z 468 ([M − NO$_3$]$^+$); elemental analysis: anal. calcd for $C_{19}H_{26}N_6O_8Zn$: C 42.91; H 4.93; N 15.80%, found: C 43.00; H 4.85; N 15.65%.

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