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Fine tuning of a solvatochromic fluorophore for selective determination of Fe^{3+}: a new type of benzimidazole-based anthracene-coupled receptor

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ABSTRACT

We synthesized a novel benzimidazole-based, anthracene-coupled fluorescent receptor capable of recognizing and estimating the concentrations of Fe^{3+} in semi-aqueous solution by ratiometric estimation. Our sensor can be made highly selective for Fe^{3+} over other metal ions by changing the solvent composition.

Fluorescence-based techniques are important tools for chemical and biochemical research because of their non-invasive nature and high intrinsic sensitivity.[1] They rely on several fundamental photophysical mechanisms including photo-induced electron transfer (PET), internal charge transfer (ICT), excimer and exciplex formations, electronic energy transfer (EET), or Förster resonance energy transfer (FRET).[2] Receptors attached to fluorescent dyes commonly employ these mechanisms for bioimaging, characterization of biological micro-structures, and quantitative estimation of analytes.[3] The methodology has rapidly progressed to meet new demands, such as analytes associated with new disease states, the need for simultaneous estimation of multiple analytes, and molecular logic gates and switches.[4]

Solvents are known to influence the physico-chemical nature of receptor binding subunits as well as the fluorescence output. Quantitative measurement of the influence of solvents on the recognition of metal ions would be greatly aided by the investigation of the solvatochromic behavior of donor–receptor units fabricated with more than one fluorophore.[5] This approach is very helpful for developing fluorescent sensor materials that exhibit changes in their fluorescent signals when exposed to different solvents and/or cations. Under present investigation, we wish to develop selective sensors for Fe^{3+} because of the role of Fe^{3+} as an important nutrient for human health and in numerous physiological functions.

We previously reported the development of benzimidazole-based receptors.[6] Here we present the synthesis of a receptor unit comprised of two fluorophores, benzimidazole, and anthracene. The solvatochromic behavior of the receptor was fine tuned to be highly selective for Fe^{3+}. The two fluorophores were chosen in such a way that both could be excited at the same wavelength, but the fluorophore output had to be different; the anthracene moiety generally exhibits 'on–off' behavior,[7] while the benzimidazole moiety may undergo both 'on–off' behavior and a charge transfer (CT) band shift.[6] This design is important for the improvement of ratiometric fluorescent recognition that measures the ratio of fluorescence intensity at two wavelengths. The ratiometric approach eliminates errors associated with receptor concentration, photobleaching, and environmental effects.[8]

Receptor **1** was synthesized as shown in Scheme 1. Compound **2** was synthesized as described in the literature.[9] Compound **4** was prepared with a 43% yield by the reaction of compound **2** with salicylaldehyde (**3**) in the presence of KOH.[10] Receptor **1** was prepared by a condensation reaction of dialdehyde **4** with 2-(2-aminophenyl)-1*H*-benzimidazole (**5**) in the presence of a catalytic amount of $Zn(ClO_4)_2$, followed by a reduction with $NaBH_4$, with a 46% yield.[11]

Receptor **1** displayed a well defined maximum at 414 nm in the fluorescence spectrum recorded at a concentration of 10 μM in pure acetonitrile, when excited at 367 nm. There were no other emission bands at any wavelengths, indicating the absence of duel channel emission due to two different fluorophores in the pure host. Sensors used in biological and environmentally important samples must be capable of monitoring analytes in aqueous media.

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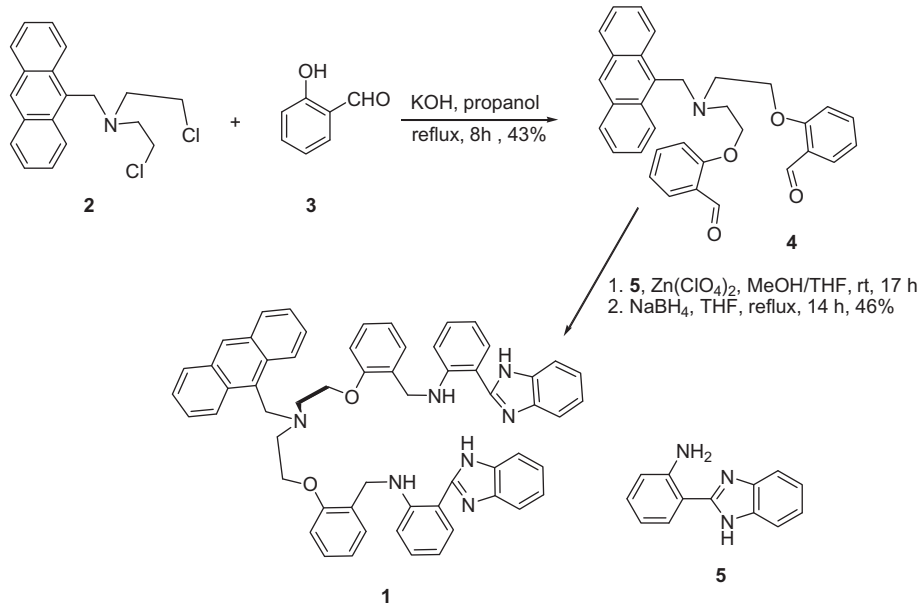

Scheme 1.

Receptor **1** was, therefore, investigated in CH_3CN/H_2O (1:1, v/v) in a HEPES-buffered solvent mixture. The sensor was excited at 367 nm at a concentration of 10 μM, and showed only one fluorescent emission band, as in acetonitrile, but with a slightly longer wavelength (424 nm).

To investigate the properties of receptor **1** as a sensor for metal ions, solutions were prepared in pure acetonitrile with a fixed concentration of receptor **1**, along with a fixed concentration of a unique metal nitrate salt. The binding of Cu^{2+} and Fe^{3+} caused a shift in the fluorescence band (Fig. 1A). This type of shift was expected because the CT band is affected when metal ions bind very closely to a fluorophore. The enhancement of fluorescence intensity upon Fe^{3+} complexation was most likely due to the cancelation of PET from nitrogen donors. On the other hand, the shift in fluorescence was due to ICT processes. Therefore, the shift and accompanying fluorescence enhancement was the consequence of both PET and ICT processes.

The different profiles of fluorescence intensity exhibited by the binding of transition metal ions prompted us to develop a sensor that was highly selective for a specific metal ion. We, therefore, studied the effect of metal-binding on fluorescence signal of receptor **1** in different solvent combinations of CH_3CN/DMSO and CH_3CN/H_2O. Receptor **1** offered high selectivity for Fe^{3+} in a solvent combination of CH_3CN/DMSO (8:2, v/v) (Fig. 1B), and presented an entirely different profile for Fe^{3+} binding in CH_3CN/H_2O (1:1, v/v; HEPES) (Fig. 1C). In this system, the emission band for metal complexation was affected by the solvent. The system thus showed solvatochromism, or the ability to change photo-physical properties due to a change in solvent polarity. The solvatochromism could be explained in terms of a positive and negative sign, which corresponds to a blue or red shift with the change in solvent polarity. This property arises due to the polarity difference between the ground state and the excited state of a fluorophore. Thus, a change in solvent polarity would lead to differential stabilization of the ground and excited states, changing the energy gap between these electronic states. Consequently, variations in the position, intensity, and shape of the spectra can be direct measures of the specific interactions between the sensor and solvent molecules.

To learn more about the properties of receptor **1** as a sensor for Fe^{3+} in CH_3CN/DMSO (8:2, v/v) and a sensor for Fe^{3+} in HEPES-buf-

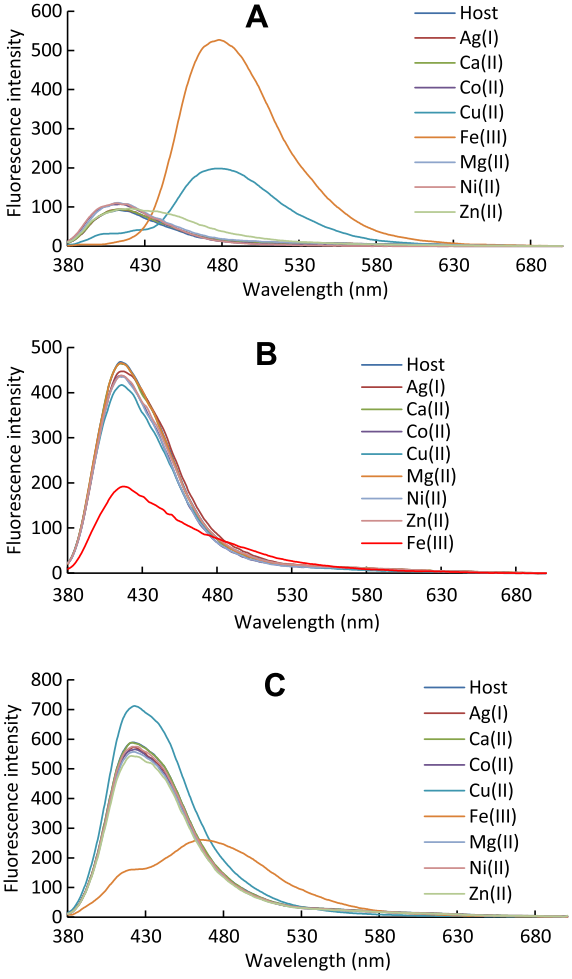

Figure 1. Changes in fluorescence intensity (λ_{ex} = 367 nm) of receptor **1** (10 μM) upon addition of a particular metal (50 μM) nitrate salt in: (A) CH_3CN; (B) CH_3CN/DMSO (8:2, v/v); (C) CH_3CN/H_2O (1:1, v/v; HEPES 100 mM; pH 7.0).

fered CH_3CN/H_2O (1:1, v/v), fluorescence titrations were performed. Figure 2 illustrates the emission response of receptor **1** with an increase in concentration of Fe^{3+} in CH_3CN/DMSO (8:2, v/v). Upon addition of Fe^{3+} to a 10 μM solution of receptor **1**, we observed a monotonic quenching of the fluorescence intensity at 424 nm and a minor enhancement was observed at 500 nm. Receptor **1** exhibited a high sensitivity toward Fe^{3+}, quenching its fluorescence intensity by 75% with 20 equiv of Fe^{3+}. We attempted to use the data for ratiometric estimation of Fe^{3+} (inset of Fig. 2) since Fe^{3+} binding leads to a change in intensity at two different wavelengths. A linear relationship was obtained within a concentration range of 100–250 μM. The association constant (K_a) of receptor **1** for Fe^{3+} was calculated on the basis of a Benesi–Hilderbrand plot (Fig. S1),[12] and was found to be $1.2 \times 10^3\ M^{-1}$. The stoichiometry of the complex was determined to be 1:1 by Job's plot (Fig. S2).[13]

Next we investigated the changes in the fluorescence spectrum of receptor **1** with an increase in concentration of Fe^{3+} in HEPES-buffered CH_3CN/H_2O (1:1, v/v) (Fig. 3). The successive addition of Fe^{3+} into a solution of receptor **1** caused a gradual quenching in fluorescence intensity at 424 nm and a prominent enhancement at 500 nm. Therefore, receptor **1** can be used for selective ratiometric estimation of Fe^{3+} along a concentration range of 0–100 μM, and the ratiometric calibration curve is shown in the inset of Figure 3. The association constant (K_a) of receptor **1** for Fe^{3+} in HEPES-buffered CH_3CN/H_2O (1:1, v/v) was calculated on the basis of a Benesi–Hilderbrand plot (Fig. S3),[12] and was found to be $4.1 \times 10^3\ M^{-1}$. The slightly lower binding strength of receptor **1** for Fe^{3+} in CH_3CN/DMSO (8:2, v/v) than in CH_3CN/H_2O (1:1, v/v) is quite expected because metal ions compete with DMSO for the binding site of receptor **1**.[14]

To judge the effect of other metal ions upon the signal response induced by Fe^{3+} complexation with receptor **1**, we carried out competitive binding experiments (Fig. 4). Fluorescence intensities were measured in a series of solutions containing receptor **1**, different amounts of Fe^{3+}, and another metal ion. The fluorescence intensity was almost identical to that obtained in the absence of any interfering metal ion. The results confirm that metal ions do not significantly influence the signal response induced by Fe^{3+} complexation with receptor **1**.

Cu^{2+} may cause potential interference in the measurement of Fe^{3+} by receptor **1** (Fig. 1C). It was thus important to test the ability of receptor **1** to operate in solutions containing equimolar concentrations of Cu^{2+} and Fe^{3+}. Figure 5 shows plots for solutions containing receptor **1** with Fe^{3+} and for those containing receptor **1** with equimolar Fe^{3+} and Cu^{2+}. The plots were in good agreement between the two sets of data. These data suggest that Fe^{3+} binding is not affected by the presence of Cu^{2+}.

The binding cavity of receptor **1** for the complexation of Fe^{3+} was assigned on the basis of MacroModel calculation (Fig. 6).[15] The calculated structure of complex shows that the sp^2 nitrogens of benzimidazole are orientated toward the binding pocket of Fe^{3+} coordination sphere. The other two nitrogens, generated through the reduction of imine linkages, also participate in binding via making a six-membered ring. The central nitrogen, which is in the vicinity to the anthracene moiety, is away from the coordination sphere. Thus, we could not find any change in the fluorescence spectrum of receptor **1** due to anthracene moiety. In addition, the calculated structure shows that the steric bulkiness of anthracene forces the two benzimidazole moieties in proximity to make an effective pseudocavity for Fe^{3+} complexation.

In conclusion, we synthesized a sensor capable of recognizing and estimating the concentrations of Fe^{3+} in semi-aqueous solution through changes in its fluorescence spectra at two different wavelengths. The sensor could be made very selective for Fe^{3+} over other metal ions by making use of the solvatochromic behavior of the receptor. Our findings provide a method of improving or altering the selectivity of known receptors by changing the solvents that can potentially lead to a new class of optical sensors.

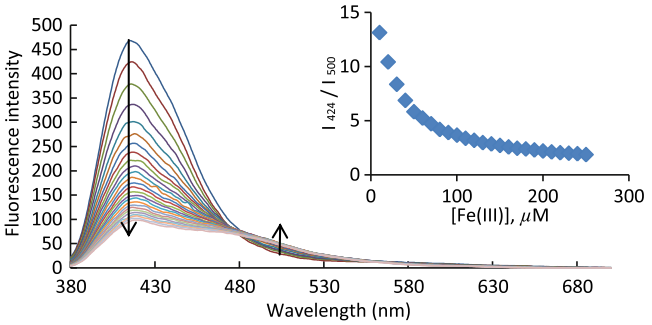

Figure 2. Fluorescence spectra changes (λ_{ex} = 367 nm) of receptor **1** (10 μM) upon addition of Fe^{3+} (0–25 equiv) as nitrate salt in CH_3CN/DMSO (8:2, v/v).

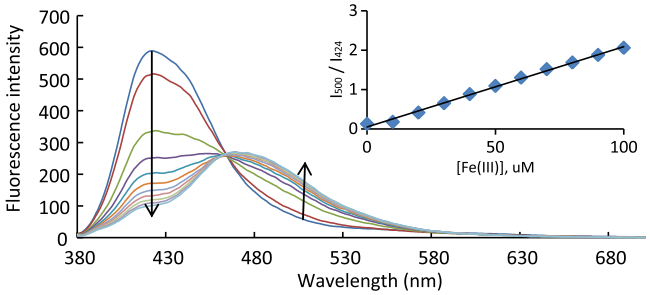

Figure 3. Fluorescence spectra changes (λ_{ex} = 367 nm) of receptor **1** (10 μM) upon addition of Fe^{3+} (0–10 equiv) as nitrate salt in HEPES-buffered CH_3CN/H_2O (1:1, v/v).

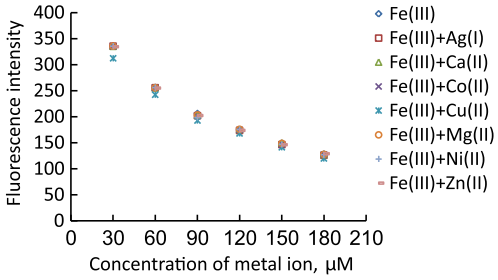

Figure 4. Fluorescence intensity (λ_{ex} = 367 nm) of receptor **1** (10 μM) upon addition of Fe^{3+} along with interfering metal nitrate salt in DMSO/H_2O (8:2, v/v) at 424 nm.

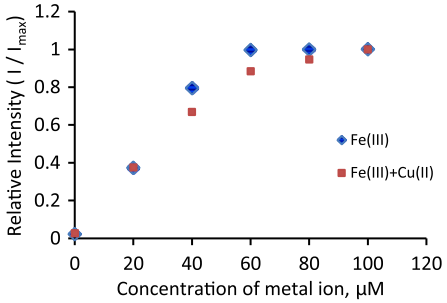

Figure 5. Plot of relative fluorescence intensity (λ_{ex} = 367 nm) of receptor **1** (10 μM) in HEPES-buffered CH_3CN/H_2O (1:1, v/v) along with blue square = [Fe^{3+}], and red square = [Fe^{3+}] + equimolar [Cu^{2+}], at 424 nm.

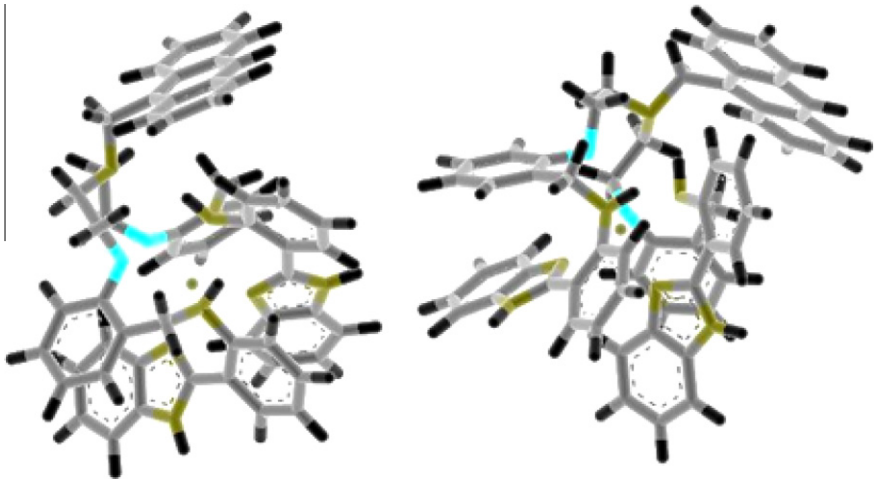

Figure 6. Energy minimized structure of Fe^{3+} complex of receptor **1** as obtained by MacroModel calculation (two different views).

Acknowledgment

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.01.077.

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10. *Synthesis of compound* **4**: light brown solid: mp 131–132 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.20 (t, 4H, $-CH_2$, J = 5.6 Hz), 4.07 (t, 4H, $-CH_2$, J = 5.6 Hz), 4.78 (s, 2H, $-CH_2$), 6.66–6.68 (m, 2H, Ar), 6.92–6.96 (m, 2H, Ar), 7.35–7.43 (m, 6H, Ar), 7.73–7.75 (m, 2H, Ar), 7.97–7.99 (m, 2H, Ar), 8.41 (s, 1H, Ar), 8.48–8.50 (m, 2H, Ar), 10.28 (s, 2H, –CHO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 52.1, 53.7, 67.5, 112.5, 121.0, 124.8, 125.1, 125.2, 126.3, 128.2, 128.7, 129.4, 131.5, 131.6, 136.0, 161.0, 189.7. Anal. Calcd for $C_{33}H_{29}NO_4$: C, 78.71; H, 5.80; N, 2.78. Found: C, 78.73; H, 5.77; N, 2.75.
11. *Synthesis of compound* **1**: light brown solid: mp 129–130 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.11 (t, 4H, $-CH_2$, J = 5.4 Hz), 4.00 (t, 4H, $-CH_2$, J = 5.4 Hz), 4.27 (s, 4H, $-CH_2$), 4.70 (s, 2H, $-CH_2$), 6.51–6.60 (m, 6H, Ar), 6.75–6.79 (m, 2H, Ar), 7.01–7.06 (m, 4H, Ar), 7.19–7.25 (m, 6H, Ar), 7.36–7.45 (m, 8H, Ar), 7.69 (s, 2H, Ar), 7.92–7.95 (m, 2H, Ar), 8.35 (s, 1H, Ar), 8.45–8.47 (m, 2H, Ar), 8.93 (br, 2H, –NH), 9.37 (br, 2H, –NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 42.6, 52.0, 53.6, 66.9, 110.9, 111.0, 112.3, 115.3, 120.6, 122.8, 125.1, 126.1, 127.0, 127.4, 128.0, 128.3, 129.2, 131.4, 131.5, 131.6, 148.3, 152.4, 156.4. Anal. Calcd for $C_{59}H_{51}N_7O_2$: C, 79.61; H, 5.78; N, 11.02. Found: C, 79.58; H, 5.77; N, 11.01.
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