A sensitive and highly selective fluorescent sensor for In^{3+}†

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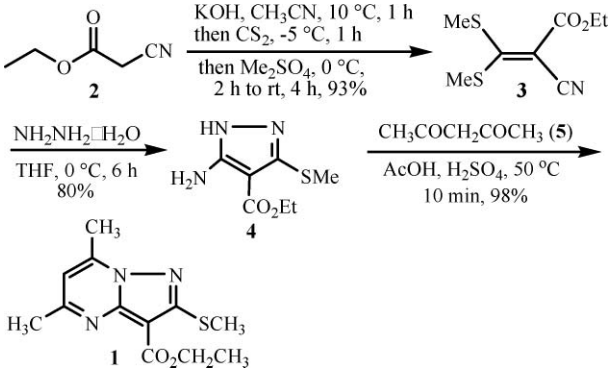
A simple neutral fluorescent sensor based on pyrazolo[1,5-*a*]pyrimidine exhibited a unique selectivity for indium(III) ion (In^{3+}) over various other metal ions with dramatic fluorescence response in acetonitrile.

Indium is an important element identified by its brilliant indigo blue flame coloration. It has significant specific applications, most notably as a component of III–V semiconductor devices, gas sensors, and transparent electrically conductive films.[1] Indium(III) salts have attracted great attentions in chemistry and biological sciences due to their water-tolerant and air-stable properties, as well as their "low toxicities".[2] However, nowadays, In^{3+} is believed to interfere with Fe^{3+} metabolism from the sites of absorption, transportation, utilization, and storage in the cells.[3] In^{3+} was found to be toxic to the liver and kidneys.[4] In^{3+} is more reactive toward biological membranes than Cd^{2+} and Hg^{2+}.[5] Moreover, In^{3+} is extremely toxic to the lungs and is a suspected carcinogen.[6] Therefore, recognition and selective sensing of In^{3+} becomes a high priority. Since fluorescent sensors are widely appreciated in the field of chemistry, biology, and environmental sciences because of their simplicity, high sensitivity and low detection limits,[7] the development of an In^{3+}-selective fluorescent sensor[8] would find applications in the study of cellular In^{3+} ion as well as in dealing with its environmental assay.[9]

During our ongoing projects aiming at the development of various functional heterocycles,[10] we found that pyrazolo[1,5-*a*]pyrimidines exhibit interesting fluorescent spectral properties.[11] Thus, we further examined the correlation between their substitution patterns and their fluorescent spectroscopic properties.[12] Complementary to these efforts, herein we report the application of pyrazolo[1,5-*a*]pyrimidine **1** (Scheme 1) as a simple, sensitive and surprisingly highly selective fluorescent sensor for In^{3+}. Pyrazolo[1,5-*a*]pyrimidine **1** was smoothly synthesized from commercially available ethyl cyanoacetate (**2**, Scheme 1). Thus, successive deprotonation of **2** with potassium hydroxide, coupling with carbon disulfide and methylation with dimethyl sulfate afforded thioacetal **3** in 93% yield. Treatment of **3** with hydrazine afforded aminopyrazole **4** in 80% yield, which was in turn converted to pyrazolo[1,5-*a*]pyrimidine **1** (98%) by condensation with pentane-2,4-dione (**5**).



Scheme 1 Synthesis of pyrazolo[1,5-*a*]pyrimidine **1**

The interaction of pyrazolo[1,5-*a*]pyrimidine **1**[13] with $In(OTf)_3$ was investigated by spectrofluorimetric titration experiments in acetonitrile (Fig. 1). The maximum excitation wavelength was selected at 309 nm according to its UV–vis spectra absorption in acetonitrile.

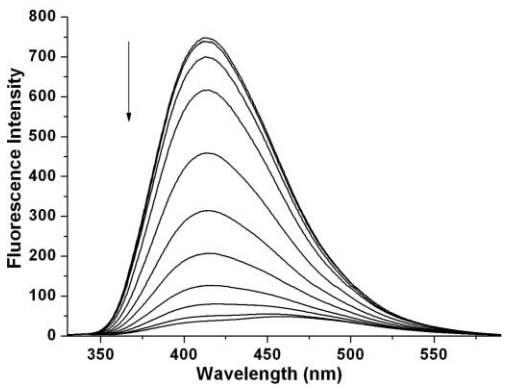


Fig. 1 Fluorescence emission spectra of pyrazolo[1,5-*a*]pyrimidine **1** (1×10^{-5} M) in the presence of $In(OTf)_3$ in CH_3CN. The concentration of In^{3+}: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0×10^{-5} M. $\lambda_{ex} = 309$ nm.

As shown in Fig. 1, pyrazolo[1,5-*a*]pyrimidine **1** shows a relatively stable emission band around 412 nm. When the concentration of In^{3+} was increased up to 1.0 molar equivalent (1×10^{-5} M), 95% quenching of the initial fluorescence of pyrazolo[1,5-*a*]pyrimidine **1** was observed. From the fluorescence titration experiments, the plotting of the change of fluorescence intensity $I_0/(I_0 - I)$ against $1/[In^{3+}]$ afforded a line with a good linear correlation coefficient ($R = 0.9988$, see ESI†), indicating the formation of a possible 1 : 1 complex. The detection limit[14] of **1** as a fluorescent sensor for the analysis of In^{3+} was determined from the plot of the fluorescence intensity as a function of the concentration of added metal ions. It was found that pyrazolo[1,5-*a*]pyrimidine **1** has a detection limit of 1.9×10^{-7} mol L^{-1} for In^{3+}, which is low

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enough for the detection of the submillimolar concentration range of In^{3+}.

In order to determine the stoichiometry of **1**-In^{3+} complex, the method of continuous variations (Job's method) was also used (Fig. 2). As expected, the result obtained from the Job plot unambiguously indicates the formation of a 1 : 1 complex between pyrazolo[1,5-*a*]pyrimidine **1** and In^{3+}.

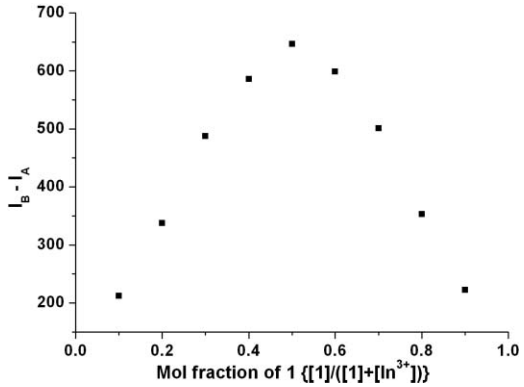

Fig. 2 Job plot between pyrazolo[1,5-*a*]pyrimidine **1** and In^{3+} in CH_3CN. [**1**] + [In^{3+}] = 2×10^{-5} M. λ_{ex} = 309 nm. I_A represents the fluorescence intensity of pyrazolo[1,5-*a*]pyrimidine **1** in the presence of In^{3+}. I_B represents the fluorescence intensity of free **1**.

The complexation of pyrazolo[1,5-*a*]pyrimidine **1** with $In(OTf)_3$ was also demonstrated by NMR titration experiments in CD_3CN (see ESI†). Partial ^1H NMR spectra of **1** in the absence and presence of In^{3+} (1.0 molar equivalent) are shown in Fig. 3.

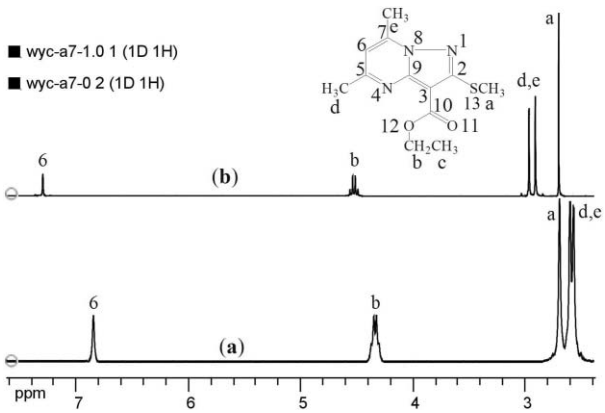

Fig. 3 (a) Partial ^1H NMR spectra of free pyrazolo[1,5-*a*]pyrimidine **1**. (b) Partial ^1H NMR spectra of **1** in the presence of $In(OTf)_3$ (1 molar equiv.).

Protons H-b adjacent to atom O-12 experience downfield shift of 0.20 ppm upon addition of In^{3+}, which indicates that In^{3+} binds with atom O-12 or O-11.[15] However, protons H-a adjacent to atom S-13 show no shift, which implies that In^{3+} has no binding with atom S-13 or N-1. The binding of In^{3+} with atom N-1 should induce the downfield shift of protons H-a by the n–π conjugation of S(13)–C(2)=N(1) bonds.[16,17] On the other hand, the formation of **1**-In^{3+} complex results in large shifts to low field of proton H-6 ($\Delta\delta$ = 0.46 ppm), protons H-d ($\Delta\delta$ = 0.34 ppm) and protons H-e ($\Delta\delta$ = 0.36 ppm), which indicates that In^{3+} complexes with the pyrimidine part. The complexation of In^{3+} with atom N-8 would lead to little downshift of protons H-d because the electronic effect (more than 3 bonds) is little and the conjugation of N(8)–C(7)=C(6)–C(5)–C(d) (single-double-single-single bonds) is inhibited. Therefore, the complexation of In^{3+} with atom N-4 should belong to the possible binding mechanism,[18] which results in the downshifts of the protons (H-6, H-d and H-e) through the σ–π hyperconjugation and π–π conjugation (Fig. 4). The similar chelating ring can be found in a reported literature,[19] where In^{3+} ion, superior to other selected metal ions, simultaneous chelates with a sp2 nitrogen and an ester oxygen to result in a five-member chelating ring. We previously believed that the possible six-member chelating ring in our case (Fig. 4) decrease the electron density at the pyrimidine part of pyrazolo[1,5-*a*]pyrimidine **1**, and thereby result in quenching of the fluorescence, which matches our previous observations that pyrazolo[1,5-*a*]pyrimidines with the pyrimidine units bearing strong electron-withdrawing groups show no fluorescence.[12] If this were indeed the case, simple protonation would equally well quench the fluorescence of pyrazolo[1,5-*a*]pyrimidine **1**. However, no significant spectral changes of pyrazolo[1,5-*a*]pyrimidine **1** occurred in the presence of 100 equivalents of acidic $^nBu_4NHSO_4$ in acetonitrile (Fig. 5). Although we are not able at this time to accurately explain the underlying mechanism of this fluorescence quenching. For these ligand donor atoms that are essentially decoupled electronically from the HOMO/LUMO residing in the chromophoric unit, one might invoke PET-type quenching. Alternatively, an inversion in the energy level of n–pi* *vs.* pi–pi* transitions and rapid ISC might explain this binding-induced quenching.

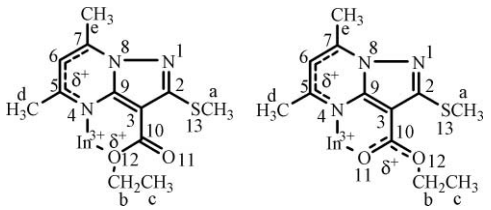

Fig. 4 Possible binding mechanisms for **1** with In^{3+}.

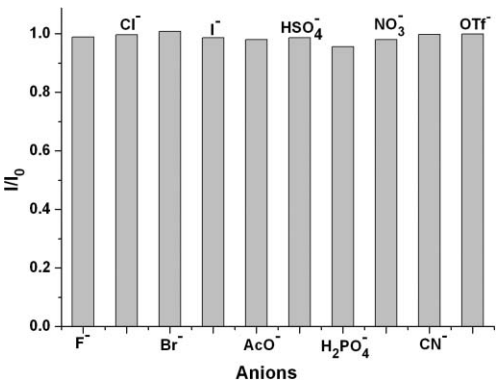

Fig. 5 Fluorescence responses of **1** in CH_3CN (1 μM) upon additions of various anions (100 μM of $^nBu_4NF$, $^nBu_4NCl$, $^nBu_4NBr$, $^nBu_4NI$, $^nBu_4NOAc$, $^nBu_4NHSO_4$, $^nBu_4NH_2PO_4$, $^nBu_4NNO_3$, $^nBu_4NCN$, $^nBu_4NOTf$). λ_{ex} = 309 nm.

To obviate the possibility that the dramatic fluorescence response of pyrazolo[1,5-*a*]pyrimidine **1** in the presence of $In(OTf)_3$

results from the formation of **1**-OTf complex. We tested the fluorescent responses of pyrazolo[1,5-*a*]pyrimidine **1** to various anions including OTf^- (Fig. 5). No significant spectral changes of pyrazolo[1,5-*a*]pyrimidine **1** occurred in the presence of 100 equivalents of F^-, Cl^-, Br^-, I^-, AcO^-, HSO_4^-, $H_2PO_4^-$, NO_3^-, CN^-, OTf^-, which indicates that pyrazolo[1,5-*a*]pyrimidine **1** shows little sensitivity towards these tested anions and the formation of **1**-In^{3+} complex leads to the corresponding fluorescence response. On the other hand, these anions, possessing different values of acidity, cause little fluorescent response of **1**, illustrating its promising application potential. In deed, $InCl_3$ and $InBr_3$, similar as the neutral salt of $In(OTf)_3$, give the similar fluorescent responses of pyrazolo[1,5-*a*]pyrimidine **1**. $In(OTf)_3$ was selected here as a representative of indium(III) salts just because most of metal salts used in our experiments are triflate salts and $In(OTf)_3$ was used for good comparisons.

With the above results in hands, we further tested the fluorescent responses of pyrazolo[1,5-*a*]pyrimidine **1** to various metal ions including common biologically available ions (see ESI†). As shown in Fig. 6 (selected representative results), no significant spectral changes of **1** occurred in the presence of 100 equivalents of Na^+(Ia), Mg^{2+}(IIa), Sc^{3+}(IIIb), Hf^{4+}(IVb), Nb^{3+}(Vb), Cr^{3+}(VIb), Mn^{2+}(VIIb), Fe^{3+}(VIIIb), Co^{2+}(VIIIb), Ni^{2+}(VIIIb), Cu^{2+}(Ib), Zn^{2+}(IIb), Al^{3+}(IIIa), Ga^{3+}(IIIa), Pb^{2+}(IVa), Bi^{3+}(Va)].

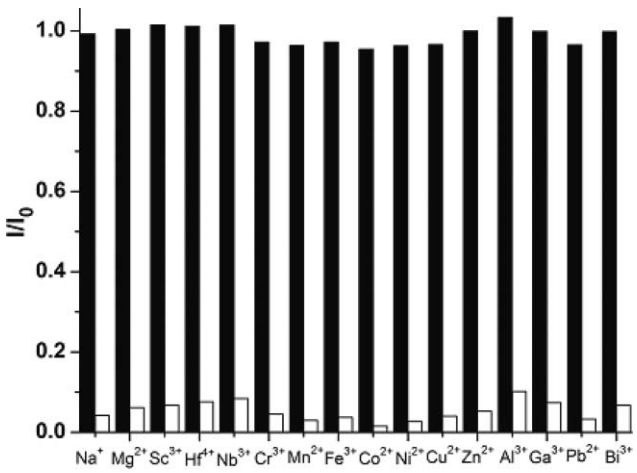

Fig. 6 Fluorescence responses of **1** to representative ions in CH_3CN (1 μM). The bars represent the ratio of the final fluorescence response (I) over the initial fluorescence response (I_0). The black bars represent the response to the addition of the given ions [20 μM of NaCl, $Mg(OTf)_2$, $Sc(OTf)_3$, $Hf(OTf)_4$, $NbBr_3$, $Cr(NO_3)_3$, $Mn(AcO)_2$, $Fe(OTf)_3$, $Co(AcO)_2$, $NiSO_4$, $Cu(OTf)_2$, $Zn(OTf)_2$, $Al(OTf)_3$, $Ga(OTf)_3$, $Pb(OAc)_2$, $Bi(OTf)_3$]. The white bars represent the response to the addition of 2 μM of In^{3+} to the respective solution. $\lambda_{ex} = 309$ nm.

These results imply that pyrazolo[1,5-*a*]pyrimidine **1** possesses good selectivity towards In^{3+}(IIIa) over various other tested metal ions. Some of these metal salts might bind with **1** since it possesses many heteroatoms (N, O and S) and unsaturated double bonds. Interestingly, the bindings do not cause significant fluorescent responses. Although unstable and/or undesired bindings might be the reasons, we are not able at this time to accurately explain the dramatic binding differences.

To test the practical applicability of pyrazolo[1,5-*a*]pyrimidine **1** as an In^{3+}-selective fluorescent sensor, competition experiments were carried on (Fig. 6).[20] Thus, pyrazolo[1,5-*a*]pyrimidine **1** (1 μM) was treated with 2 μM of In^{3+} in the presence of background metal ions (20 μM), respectively. These background metal ions have no significant interference with the detection of In^{3+}, which suggests that pyrazolo[1,5-*a*]pyrimidine **1** has potential to be used as an In^{3+}-selective fluorescent chemosensor.

In summary, we present pyrazolo[1,5-*a*]pyrimidine **1** as a promising fluorescent sensor for the fluorimetric In^{3+} detection, which shows high sensitivity and selectivity toward In^{3+} over a wide range of metal ions in acetonitile. Its binding properties have been extensively studied mainly by spectrofluorimetric titration experiments, Job's method, NMR titration experiments and competition experiments. These experiments were performed in anhydrous acetonitrile. When aqueous solutions of In^{3+} salts were added to the acetonitrile solution of pyrazolo[1,5-*a*]pyrimidine **1**, desired results could also be obtained by drying the system over anhydrous sodium sulfate. Studies on making new derivatives of the heterocycle suitable for aqueous use are in progress. We envision that this kind of In^{3+}-selective fluorescent sensors could find practical applications in the future.

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