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A distyryl BODIPY derivative as a fluorescent probe for selective detection of chromium(III)

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ABSTRACT

A new boradiazaindacene (BODIPY) derivative (**1a**) bearing simple NO bidentate ligands has been synthesized. The **1a** molecule behaves as a fluorescent probe for Cr^{3+} and shows shows a strong red fluorescence upon coordination with Cr^{3+}, while showing almost no fluorescence for other metal cations.

Trivalent chromium (Cr^{3+}) is an essential nutrient for humans and plays an important role in the metabolism of carbohydrates, lipids, proteins, and nucleic acids.[1] Insufficient intake of Cr^{3+} increases the risk for diabetes and cardiovascular diseases, whereas excessive intake causes genotoxic effects.[2] Accurate and rapid determination of Cr^{3+} amount in the environment is therefore necessary. Traditional analytical methods for Cr^{3+} employ expensive instruments such as atomic absorption spectrometry[3] and inductively-coupled plasma atomic emission spectrometry.[4] Although these methods enable accurate and selective detection of Cr^{3+}, these are usually time-consuming and require complicated and tedious sample preparation.

Fluorescence analysis with probe molecules is one of the alternative methods because it enables easy, simple, and rapid analysis of metal cations with inexpensive instruments.[5] Several kinds of fluorescent probes have been proposed so far, but there are only five reports of Cr^{3+} probe.[6] In addition, these probes show poor selectivity for Cr^{3+}; the fluorescence intensity obtained with Cr^{3+} is four times less than that obtained with other metal cations.

BODIPY is a dye which has been studied extensively for application to various kinds of functional materials because of its high fluorescence quantum yields and large extinction coefficients.[7] Several BODIPY-based fluorescent probes have been proposed so far for the detection of various analytes such as cations, anions, and molecules.[7] Of recent particular interest is the synthesis of BODIPY-based probes that show absorption and emission in red visible or near infrared (NIR) region.[8] This is because long wavelength light is beneficial for intracellular fluorescence imaging due to low light scattering and low background signal.[9]

In the present work, we synthesized a BODIPY-based fluorescent probe containing simple NO bidentate ligands, **1a** (Scheme 1). Upon addition of Cr^{3+}, the probe shows a strong fluorescence in the red visible region (610–720 nm). The Cr^{3+} selectivity of the probe is much higher than the early reported probes and thus allows selective Cr^{3+} detection.

The distyryl probe, **1a**, was synthesized via three steps according to the procedure summarized in Scheme 1 with 66% overall yield (see Synthesis, Supplementary data). A Knoevenagel-type condensation of a BODIPY derivative, **2**,[10] with 4-acetamidobenzaldehyde affords a distyryl BODIPY compound, **3a**, in 91% yield. Deprotection of the acetyl groups of **3a** affords **4a** in 97% yield. Condensation of **4a** with salicylaldehyde followed by reduction with $NaBH_4$ gives rise to the probe, **1a**, in 75% yield. To clarify the coordination and sensing properties of **1a**, a monostyryl probe, **1b**, was also synthesized in a similar manner to **1a** with 15% overall yield. The structures of these probes were fully characterized by ^1H NMR, ^{13}C NMR, MS, and HRMS analysis (Figs. S1–S12, Supplementary data).

Figure 1a shows the fluorescence spectra (λ_{ex} = 560 nm) of **1a** (5 μM) measured in CH_3CN with 40 equiv of respective metal cations. Without cations, **1a** shows almost no fluorescence, where the fluorescence quantum yield (Φ_F) is determined to be 0.003.[11] This is because, as usually observed,[5c] electron transfer from the amine nitrogens to the photoexcited BODIPY moiety quenches the fluorescence. Addition of Cr^{3+}, however, creates a strong fluorescence (Φ_F = 0.69) in the red visible region at 610–720 nm with an emission maximum at 643 nm. As shown in the inset picture, bright

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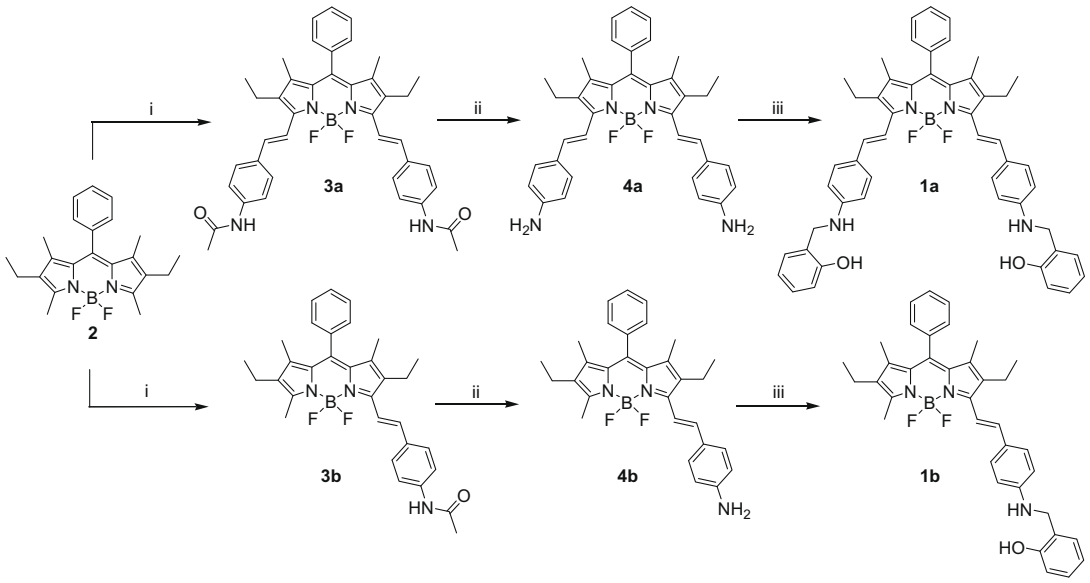


Scheme 1. Reagents and conditions: (i) 4-acetamidobenzaldehyde, piperidine, acetic acid, reflux, overnight; (ii) (1) 1 M HCl/MeOH, 100 °C, 6 h; (2) Et_3N, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 1 h; (iii) (1) salicylaldehyde, CH_2Cl_2/MeOH, rt; (2) $NaBH_4$, rt, 5 min.

red fluorescence is observed upon Cr^{3+} addition. In contrast, addition of other metal cations scarcely shows fluorescence (Φ_F <0.01). Figure 1b shows the fluorescence enhancement factor (F/F_0) defined as the ratio of the fluorescence intensity measured with and without metal cations. With Cr^{3+}, the enhancement factor is determined to be 2870. In contrast, the factors for other cations are less than 70. This suggests that the factor for Cr^{3+} is more than 40 times larger than that for other metal cations, which is much larger than the value obtained with early reported Cr^{3+} probes (<4 times).[6] The above-mentioned findings clearly indicate that **1a** behaves as a highly selective fluorescent Cr^{3+} probe.

As shown in Figure S13 (Supplementary data), the monostyryl BODIPY derivative, **1b**, also shows almost no fluorescence without cations, but addition of Cr^{3+} creates a strong fluorescence (enhancement factor: 1380), as does **1a**. However, **1b** shows a fluorescence enhancement upon the addition of Hg^{2+} (enhancement factor: 844) and Fe^{2+} (410). This indicates that Cr^{3+} selectivity of **1b** is poor and two NO bidentate ligands for **1a** are necessary for selective Cr^{3+} detection.

Figure 2 shows the result of fluorescence titration of **1a** with Cr^{3+}. The stepwise Cr^{3+} addition leads to an increase in the 643 nm fluorescence, and the increase is saturated upon the addition of 40 equiv of Cr^{3+}. The fluorescence increase takes place immediately after Cr^{3+} addition (within 10 s), indicating that **1a** enables rapid detection of Cr^{3+}.

Figure 3a shows the result of absorption titration of **1a** with Cr^{3+}. Without cations, **1a** shows an intense absorption band centered at 692 nm (ε 57,100 $M^{-1}\,cm^{-1}$). Upon the addition of Cr^{3+}, the 692 nm band decreases and a blue-shifted band appears at 628 nm (ε 64,300 $M^{-1}\,cm^{-1}$). As shown in the inset picture, the solution color changes from green to blue upon Cr^{3+} addition. As shown in Figure 3b, the spectral change almost stops upon the addition of 40 equiv of Cr^{3+}, which is similar to the fluorescence titration result (Fig. 2b).

As shown in Figure S15 (Supplementary data), absorption titration of the monostyryl BODIPY derivative, **1b**, with Cr^{3+} also shows a blue shift of the absorption band (603–573 nm), as is the case for **1a**. During the spectral change of **1b**, a clear isosbestic point is observed at 581 nm, indicating that coordination of **1b** with Cr^{3+} produces a single component. As reported,[6b,c,12] a N_2O_2 tetradentate ligand usually coordinates with Cr^{3+} in a 1:1 stoichiometry. This implies that two **1b** molecules are involved in the coordination with Cr^{3+} and produce a 2:1 complex.

In contrast, coordination of **1a** with Cr^{3+} produces two kinds of complexes; a 2:1 complex is produced at low Cr^{3+} amount, but further Cr^{3+} addition leads to a transformation into a 2:2 complex via sequential coordination with another Cr^{3+}. As shown in Figure 3a, there is no isosbestic point observed throughout the titration with 0–40 equiv of Cr^{3+}. Detailed titration results (Fig. S16, Supplementary data), however, reveal that two isosbestic points exist at 674 nm in the range of 0–2 equiv of Cr^{3+} and at 643 nm in the range of 7–40 equiv of Cr^{3+}, respectively, whereas no isosbestic point is observed at 2–7 equiv of Cr^{3+}. The coordination sequence of **1a** with Cr^{3+} can be explained to be analogous fashion to that of **1b**, as shown in Scheme 2. The 674 nm isosbestic point is due to the formation of a 2:1 complex. In contrast, the 643 nm isosbestic point is due to the transformation of the complex to a 2:2 complex via a coordination with another Cr^{3+}. The absence of isosbestic point at 2–7 equiv of Cr^{3+} is because all three species (free **1a**, 2:1 complex, and 2:2 complex) exist in solution at once. At the present stage, we have not obtained direct evidence of the formation of 2:1 and 2:2 complexes; ESI-MS analysis of a CH_3CN solution containing **1a** and Cr^{3+} does not show clear mass assigned to the complexes, and Job's plot analysis (Fig. S17, Supplementary data) does not provide clear stoichiometry. These are probably because of a weak binding affinity between **1a** and Cr^{3+}. However, the isosbestic points observed during the titration of **1a** (Fig. 3a) support the coordination sequence of **1a** shown in Scheme 2.

As shown in Figure 2b, fluorescence intensity enhancement is very weak at 0–2 equiv of Cr^{3+} where the 2:1 complex exists mainly. In contrast, strong fluorescence enhancement is observed at higher Cr^{3+} concentration where the 2:2 complex exists mainly. The weak fluorescence of the 2:1 complex is probably because the uncoordinated amine nitrogens of **1a** quench the fluorescence via an electron transfer to the photoexcited BODIPY moieties.[5c] This leaves that the 2:2 complex is the major emitting species in the present system.

Figure 4 shows the effect of water addition on the Cr^{3+}-induced fluorescence enhancement of **1a**. The fluorescence intensity measured with 40 equiv of Cr^{3+} decreases with an increase in the water

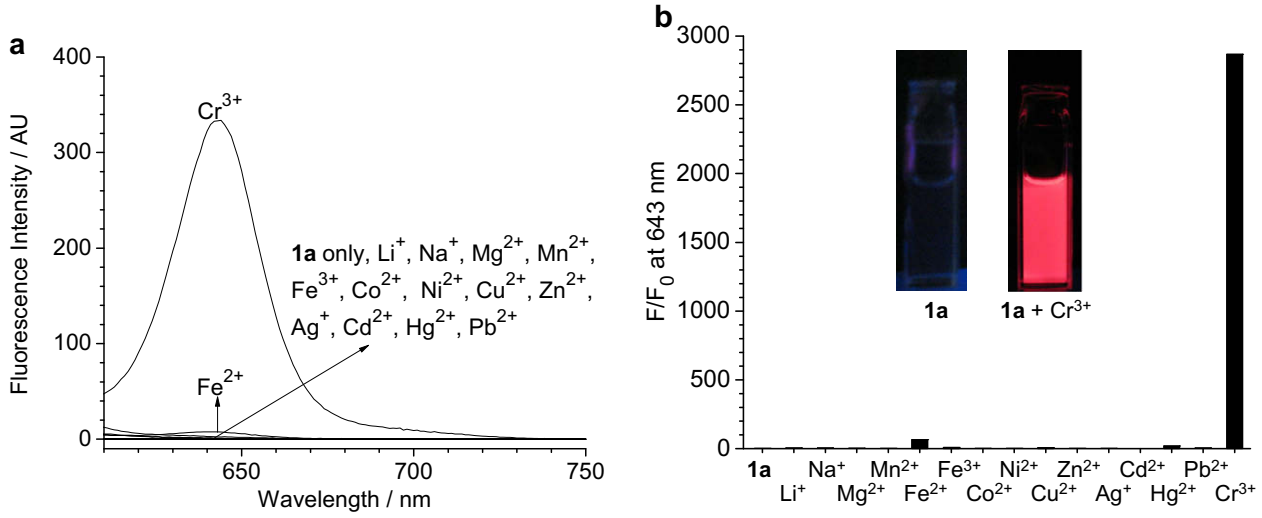


Figure 1. (a) Fluorescence spectra (λ_{ex} = 560 nm) of **1a** (5 μM) measured in CH_3CN with 40 equiv of respective metal cations. (b) Fluorescence enhancement factor (F/F_0), where F and F_0 are the fluorescence intensity measured at 643 nm with and without metal cations, respectively. (Inset) Change in fluorescence color of the solution upon Cr^{3+} addition.

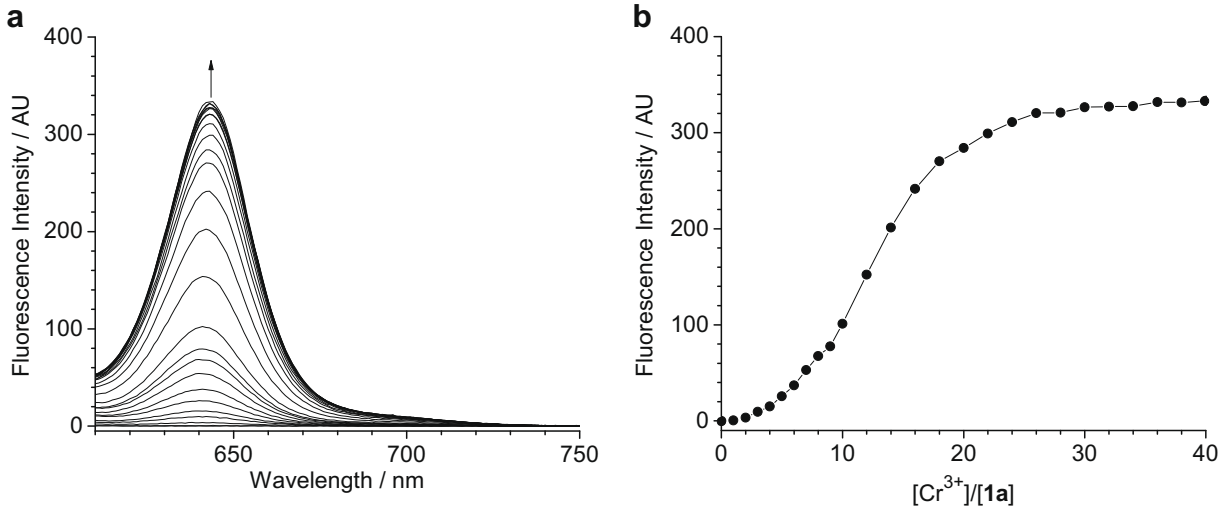


Figure 2. (a) Change in fluorescence spectra (λ_{ex} = 560 nm) of **1a** (5 μM) in CH_3CN upon addition of Cr^{3+}, where the Cr^{3+} amount is 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 and 40 equiv. (b) Change in fluorescence intensity monitored at 643 nm.

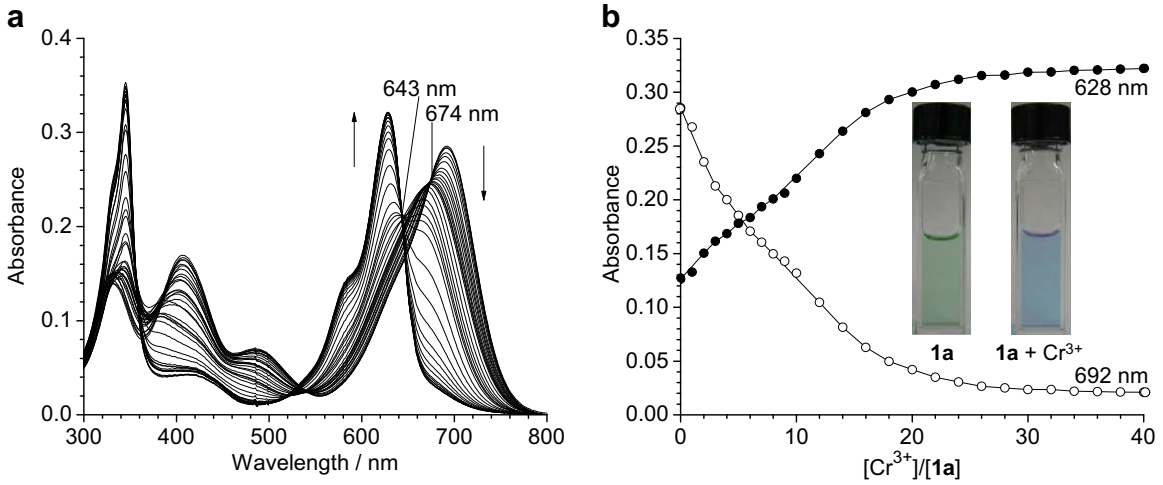
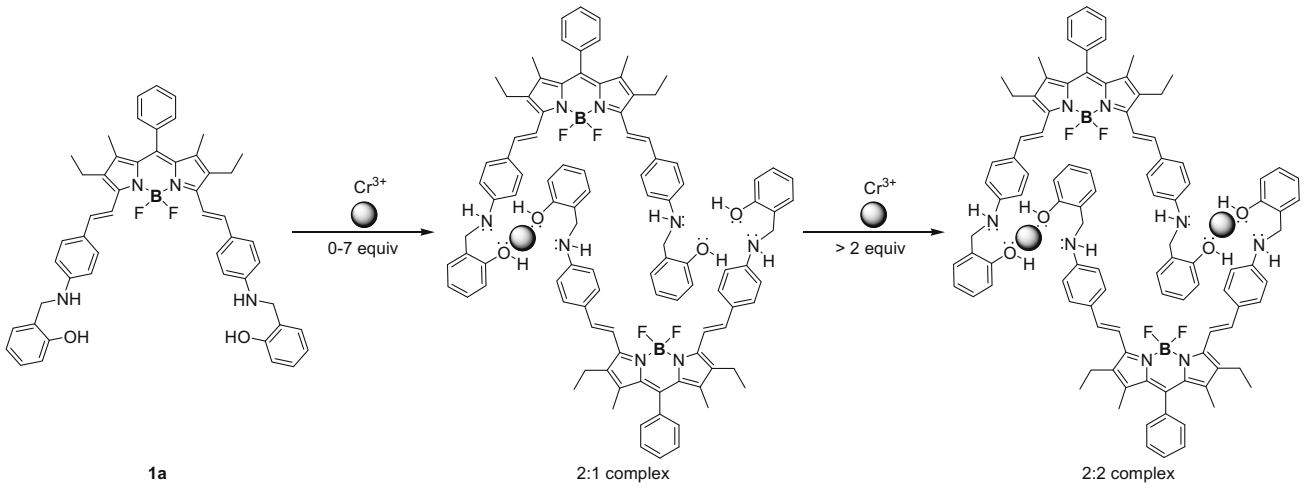


Figure 3. (a) Change in absorption spectra of **1a** (5 μM) in CH_3CN upon addition of Cr^{3+}, where the Cr^{3+} amount is 0, 0.2, 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4, 2.6, 2.8, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38 and 40 equiv. (b) Change in absorbance at 692 nm and 628 nm. (Insert) Color change of the solution upon Cr^{3+} addition. The change in 3D absorption spectra upon Cr^{3+} addition is shown in Figure S14 (Supplementary data).



Scheme 2. A possible sequence for coordination between **1a** and Cr^{3+}.

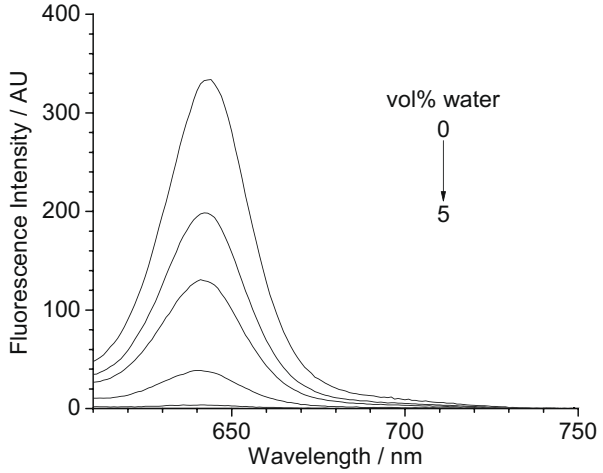


Figure 4. Fluorescence spectra (λ_{ex} = 560 nm) of **1a** (5 μM) measured with 40 equiv of Cr^{3+} in CH_3CN in the presence of 0, 0.5%, 1%, 2% and 5% water.

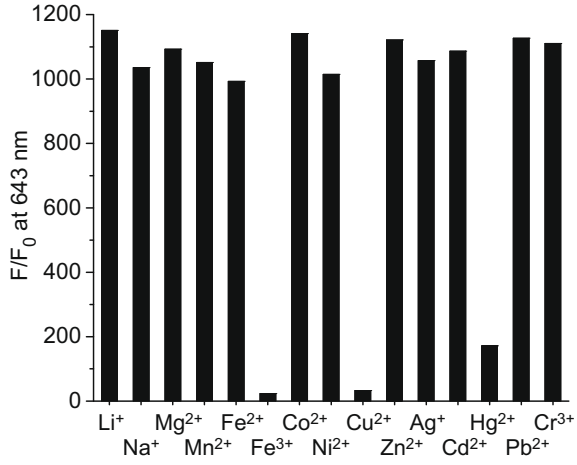


Figure 5. Fluorescence enhancement factor (F/F_0) of **1a** (5 μM) measured in CH_3CN containing 1% water with 40 equiv of Cr^{3+} and 40 equiv of other respective metal cations. F and F_0 are the fluorescence intensity monitored at 643 nm with and without cations, respectively.

content, where addition of 5% water leads to a complete quenching. This is because water suppresses the coordination between NO ligands with Cr^{3+}. However, with 1% water, strong fluorescence still remains (enhancement factor: 1110), indicating that the probe **1a** allows the fluorometric detection of Cr^{3+} for samples containing less than 1% water.

The probe **1a** enables selective detection of Cr^{3+} even in the presence of many other metal cations. Figure 5 shows the fluorescence enhancement factor (F/F_0) measured with Cr^{3+} (40 equiv) and other metal cation (40 equiv) in CH_3CN containing 1% water. The Cr^{3+}-induced fluorescence enhancement is unaffected in the presence of many other cations (Li^+, Na^+, Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ag^+, Cd^{2+}, and Pb^{2+}). In contrast, some cations such as Fe^{3+}, Cu^{2+}, and Hg^{2+} strongly quench the fluorescence. As shown in Figure S18 (Supplementary data), addition of these three cations leads to a significant blue shift of the absorption spectra of **1a** to 450–580 nm even in the presence of Cr^{3+}, which is much shorter than that obtained only with Cr^{3+} (550–720 nm). This clearly indicates that **1a** coordinates with these cations (Fe^{3+}, Cu^{2+}, and Hg^{2+}) more strongly than Cr^{3+}. This suppresses the coordination with Cr^{3+} and, hence, results in almost no fluorescence enhancement.

In summary, we found that a new distyryl BODIPY derivative, **1a**, behaves as a selective and red-emitting Cr^{3+} probe. The probe allows selective Cr^{3+} detection in the presence of contaminating metal cations other than Fe^{3+}, Cu^{2+}, and Hg^{2+}. Detailed coordination structure between **1a** and Cr^{3+} still remains to be clarified. In addition, there are several problems for practical application of the probe.[13] However, the simple molecular design presented here using a NO bidentate ligand may contribute to the development of more efficient and more useful fluorescent probe for Cr^{3+} detection.

Acknowledgments

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Supplementary data

Supplementary data (general methods, synthesis, Figure S1–S18) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.03.013.

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11. Fluorescence quantum yield was determined with rhodamine B as a standard (Φ_F = 0.69 in ethanol): Parker, C. A.; Rees, W. T. *Analyst* **1960**, *85*, 587–600.
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13. A referee pointed out that the probe works in solution with <1% water. In addition, the Cr^{3+}-induced fluorescence is quenched by some metal cations (Cu^{2+}, Hg^{2+}, and Fe^{3+}). These are the critical problems for practical application. However, the probe shows a selective, strong, and red fluorescence for Cr^{3+}. In addition, the emission enhancement factor of the probe for Cr^{3+} as compared to other cations is much higher than that of early reported probes. We therefore strongly believe that the results presented here provide important information for the development of Cr^{3+}-selective practical probes.