A Luminescent Metalloreceptor Exhibiting Remarkably High Selectivity for Mg^{2+} over Ca^{2+}

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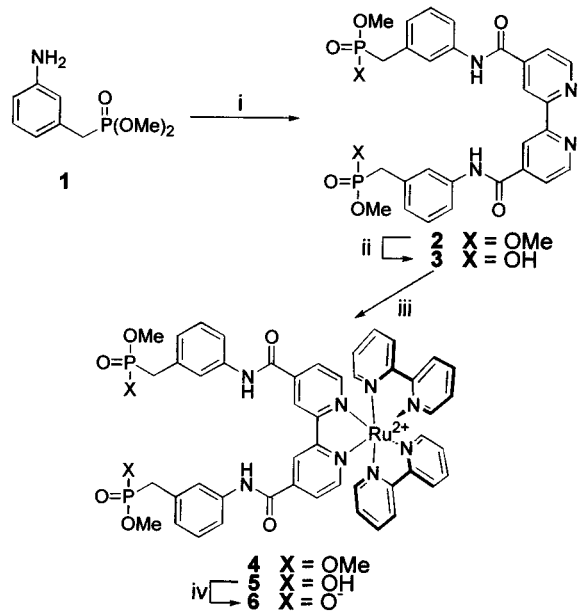
Fluorescence spectroscopy is the most powerful research tool for visualizing the structure and dynamics of living systems at the molecular level.[1−4] During the last two decades, extensive efforts have been exerted to develop fluorescent probes for biologically relevant ions and molecules.[5−7] Effective intracellular probes for alkali and alkali earth metal ions are commercially available at present.[8] However, there is still a need for probes with an improved specific response, in particular, in binding selectivity for Mg^{2+} over Ca^{2+}; under high calcium concentrations, it is often difficult to discriminate the effects of Mg^{2+} and Ca^{2+} because almost all of the current Mg^{2+} probes are a variant of the Ca^{2+} probes, which respond to these two cations with identical spectral changes.[9] Numerous approaches to enhance the Mg^{2+}/Ca^{2+} selectivity have predominantly exploited differences in the size of these cations, the so-called "size selectivity". To find another possible way to discriminate between Mg^{2+} and Ca^{2+}, we have designed an induced-fit type of cation chelator in which, upon approach of a desired cation, the binding site can be selectively organized against the electrostatic repulsion of two negative ligands. Here we report the synthesis and optical ion-sensing properties of a metalloreceptor **6** (Figure 2), in which the induced-fit chelator is incorporated into a luminescent tris-(bipyridyl) ruthenium complex.

The synthesis of **6** is summarized in Scheme 1. The starting aniline **1** was prepared by reaction of *m*-nitrobenzyl bromide and trimethyl phosphite at 100 °C for 2 h, followed by reduction with $NaBH_4/NiCl_2$ in 77% overall yield. Coupling of **1** and 4,4′-bis-(chlorocarbonyl)-2,2′-bipyridine[10] in *N*,*N*′-dimethylacetamide (DMAC) gave **2** in 65% yield. Demethylation of **2** with *N*-methylmorpholine gave **3** in 98% yield. Condensation of **3** with $Ru(bpy)_2Cl_2$[11] (bpy = 2,2′-bipyridine) followed by addition of NH_4PF_6 gave **5** in 92% yield. The acid form **5** was converted to a deprotonated form by treating with 10% aqueous Et_4NOH, and then its counterions were removed by washing with organic solvents to give free zwitterionic metalloreceptor **6** in 91% yield. A metalloreceptor **4**, which does not have negatively charged ligands, was also prepared in analogy to **5**, starting from **2**.[12]

Absorption and luminescence titration of **6** with alkali (Li^+, Na^+, K^+) and alkali earth (Mg^{2+}, Ca^{2+}, Ba^{2+}) metal ions were preliminarily performed in DMSO at 20 °C. Upon addition of these cations except for Mg^{2+}, **6** showed no change in absorption and luminescence spectra. In contrast, the addition of Mg^{2+} led to a blue-shift of the MLCT absorption band with clear isosbestic points at 489, 422, and 368 nm (Figure 1a). The luminescence of **6** was also affected by addition of Mg^{2+}, significantly increasing the MLCT emission band with a slight blue-shift (5 nm) as shown in Figure 1b. Thus, the optical response of **6** for cation binding is highly selective for Mg^{2+}. Titration of **4** with the cations was also carried out under the same conditions. The absorption and luminescence spectra of **4** showed no change upon addition of any cations. Electrostatic attraction between Mg^{2+} and negative phosphinates is responsible for the cation binding observed in **6**.

It is interesting to note that, despite the flexible binding site, **6** exhibits perfect selectivity for Mg^{2+} over Ca^{2+}. The complexation process was further studied by ^1H NMR spectroscopy. When 0−5 equiv of Mg^{2+} was added to a 3×10^{-3} M solution of **6** in DMSO-d_6, the most pronounced upfield shift (>0.5 ppm) occurred

Scheme 1[a]



[a] i, 4,4′-bis(chlorocarbonyl)-2,2′-bipyridine,[10] Et_3N, DMAC, rt, 12 h; ii, *N*-methylmorpholine, 20% DMAC/MeOH, 70 °C, 7 days; iii, $Ru(bpy)_2Cl_2$, 50% EtOH, reflux, NH_4PF_6; iv, 10% aq Et_4NOH, EtOH.

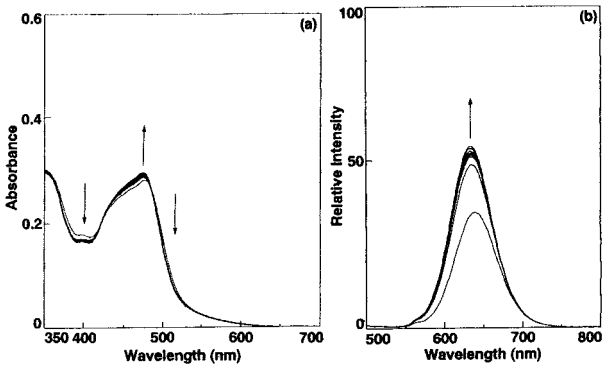


Figure 1. Change in the UV−vis absorption and luminescence spectra of **6** upon addition of $Mg(ClO_4)_2$ in DMSO at 293K: **[6]** = 2.0×10^{-5} M, **[Mg^{2+}]** = $0-6.0 \times 10^{-4}$ M. Excitation was at 489 nm.

(1) *Molecular Luminescence Spectroscopy Methods and Applications: Part 1*; Schulman, S. G., Ed.; John Wily & Sons: New York, 1985.
(2) *Topics in Fluorescence Spectroscopy*; Lakowicz, J. R., Ed.; Plenum Press: New York, 1991; Vol. 3.
(3) *Fluorescence Spectroscopy: New Methods and Applications*; Wolfbeis, O. S., Ed.; Springer-Verlag: New York, 1993.
(4) *Fluorescence Spectroscopy*; Brand, L., Johnson, M. L., Eds.; Academic Press: San Diego, 1997.
(5) *Fluorescent and Luminescent Probes for Biological Activity*; Wason, W. T., Ed.; Academic Press: San Diego, 1993.
(6) Slavik, J. *Fluorescent Probes in Cellular and Molecular Biology*; CRC Press: Boca Raton, 1993.
(7) *Imaging Living Cells*; Rizzuto, R., Fasolato, C., Eds.; Springer: Berlin, 1999.
(8) *Handbook of Fluorescent Probes and Research Chemicals*; Spence, M. T. Z., Ed.; Molecular Probes: Eugene, OR, 1996.
(9) Hurley, T. W.; Ryan, M. P.; Brinck, R. W. *Am. J. Physiol.* **1992**, *263*, C300.
(10) Whittle, C. P. *J. Heterocycl. Chem.* **1977**, *14*, 191.
(11) Sullivan, B. P.; Salmon, D. J.; Meyer, T. J. *Inorg. Chem.* **1978**. *17*, 3334.

(12) All new compounds have been characterized by IR, ^1H NMR, and mass spectrometry, and give satisfactory elemental analyses. The experimental details will be reported elsewhere.

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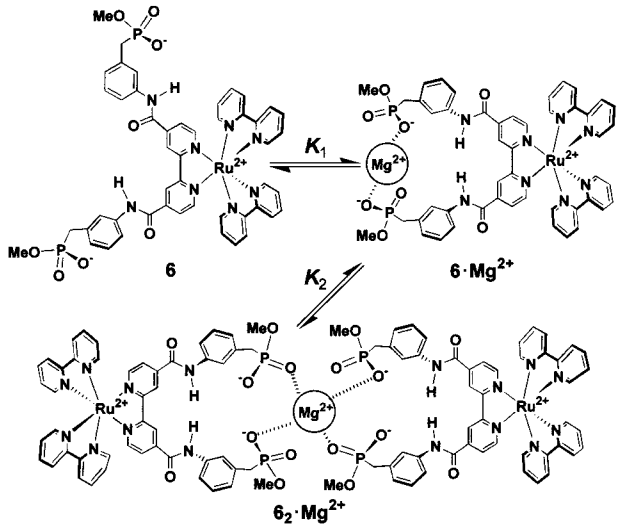

Figure 2. Proposed structure for the 1:1 and 2:1 complexes formed between **6** and Mg^{2+}.

in the P−OCH_3 protons. The significant downshift was also observed in the P−CH_2− protons. These spectral changes indicate the coordination of Mg^{2+} to the phosphinate groups. In addition, the phenyl and 3,3′-bipyridyl protons showed distinct chemical shift changes. There was a marked tendency for the resonances to saturate before 1 equiv of Mg^{2+} was added to the solution. These results suggest multiple equilibria with the possible binding of Mg^{2+} with two and more metalloreceptors **6**. Figure 2 shows possible binding geometries for the 1:1 and 2:1 complexes. The complex formation is accompanied with a dramatic structural rearrangement of the binding site. Absorption and luminescence titrations described above clearly indicate that Mg^{2+} can selectively organize the binding site to the optimal binding geometry. As the highly flexible binding site of **6** can accommodate all of the cations tested, the high preference for Mg^{2+} cannot be explained by "size selectivity." As shown in Figure 2, the negative phosphinates need to come close to the coordination position upon complexation. The charge densities [13] of the metal ions increase in following order: K^+ (0.05) < Na^+ (0.10) < Ba^{2+} (0.13) < Ca^{2+} (0.24) < Li^+ (0.33) ≪ Mg^{2+} (0.75). Magnesium cation has the highest charge density in a series of metal ions, which is most suitable to organize the binding pocket in the face of the electrostatic repulsion of the negative phosphinates.

To explore solvent effects on the high Mg^{2+} selectivity of **6**, binding and optical sensing of metal ions were examined in different ratios of H_2O/DMSO. In percentages between 0 and 15, **6** responded strongly and selectively to the presence of Mg^{2+}. As H_2O percentages approached and exceeded 15, 1:1 and 2:1 complexes of **6** and Mg^{2+}were dominantly formed. At 15% H_2O/DMSO, binding constants K_1 and K_2 for Mg^{2+} were calculated from the titration data using Hyperquad2000 computer program[14] to be 19800 ± 3400 and 1800 ± 760 M^{-1}, respectively. An 80% increase in the emission intensity was observed over the 0−0.5 mM Mg^{2+} concentration range (see Supporting Information S4 and S7). At 20−25% H_2O, the luminescence enhancement became negligible (<5%). The complexes were too weak to estimate the accurate value of K_a's. However, the high selectivity for Mg^{2+} was still observed even in 25% H_2O/DMSO. The enhanced emission intensity can be explained by the induced-fit complexation that decreases the high conformational flexibility of **6**, and thereby nonradiative decay processes of vibrational and rotational relaxation are inhibited.

Although the binding nature of **6** is still not completely elucidated, it is clear from the present study that "size selectivity" is not only the way to distinguish between Mg^{2+} and Ca^{2+}. The highly selective optical sensing of Mg^{2+} would be attainable by using an appropriate induced-fit receptor of phosphinate−luminophore conjugate. We are continuing our work on this topic.

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Supporting Information Available: Binding details and ^{1}H NMR, UV−vis, and luminescence titration data of **6** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The charge density (ρ) is defined as the amount of electric charge per unit volume. It is here given by $\rho = q/(4/3\pi r^3)$ where q is the formal charge (+1 or +2) and r is Shannon ionic radius. The following values of r (Å) were used: Li^+ (0.90), Na^+ (1.32), K^+ (1.65), Mg^{2+} (0.86), Ca^{2+} (1.26), Ba^{2+} (1.56). Shannon, R. D. *Acta Crystallogr.* **1976**, *A32*, 751.

(14) Gans, P.: Sabatini, A.: Vacca, A. *Talanta* **1996**, *43*, 1739.