Molecular Recognition

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A Porphyrin-Related Macrocycle with an Embedded 1,10-Phenanthroline Moiety: Fluorescent Magnesium(II) Ion Sensor**

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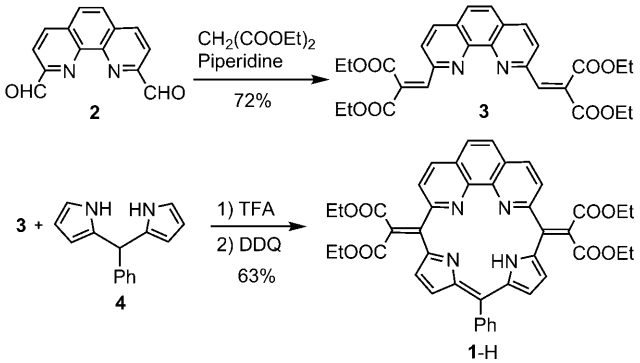
Fluorescent sensors for various metal ions have attracted much interest because of their convenient use and high sensitivity, and they have been employed in efforts to clarify the real-time dynamics and various biological functions of targeted metal cations in living cells.[1] Selective detection and quantification of biologically important cations such as Na^+, K^+, Mg^{2+}, and Ca^{2+} is of great importance for understanding cell physiology.[2] The Mg^{2+} ion is one of the most abundant divalent cations in cells. Intracellular Mg^{2+} plays critical roles, for example, as an enzyme cofactor, a DNA conformation stabilizer, a facilitator of transmembrane ion transport, and as an effector of signal transduction.[3]

Recently, various families of Mg^{2+}-responsive fluorescent probes have been developed. These probes have receptor groups based upon moieties including a charged β diketone,[4] diaza-18-crown-6,[5] benzo-15-crown-5,[6] calix[4]arene,[7] and imine-linked aromatics.[8] Some examples of these probes have been utilized in biological applications.

It would be desirable to extend the family of fluorescent sensors for Mg^{2+} to those capable of emitting longer wavelength fluorescence. Porphyrins and their derivatives, which possess both a fluorophore and a coordination platform, are expected to be attractive candidates for the development of cation-responsive receptors. These molecules have ideal properties such as a cell-penetrating far-red or near IR fluorescent emission, as well as large stokes shifts which minimize the effects of the background fluorescence.[9] Although a large number of porphyrin analogues[10] (e.g. sapphyrins, rubyrins) have been employed in recognition and sensing for various kind of anions, reports on the use of such porphyrins in the field of cation sensing are quite rare.[11] This rarity is most likely a result of slow metalation rates and low association constants.[12] Especially, Mg–porphyrin complexes are less stable divalent metal complexes as judged from the stability index reported by Buchler[14] for a Mg–porphyrin complex ($Si = 3.64$).

To overcome these problems, we have designed a novel porphyrin-related macrocycle containing key structural improvements to enable facile coordination of Mg^{2+}: 1) a nonpolar distorted macrocycle, which decreases the barrier for the formation of the first bond to the pyrrolic nitrogen atom; 2) a smaller coordination environment in the cavity, which is suitable for accommodation of the smaller Mg^{2+} relative to other divalent cations (ionic radius: 0.72 Å), and 3) the presence of one proton-bearing nitrogen atom, relative to two found in a porphyrin, minimizes the steps needed for complex formation with a metal. In accord with these features, we designed and synthesized a novel porphyrin analogue, **1**-H, which has a 1,10-phenanthroline moiety embedded within the macrocycle. 1,10-Phenanthroline provides two juxtaposed neutral nitrogen donor sites. This entropic advantage ensures rapid complexation of metal ions. Furthermore, the distance between the juxtaposed nitrogen atoms is shorter relative to those of porphyrins. This provides a smaller coordination site for metal ions in the central cavity of the porphyrin.[15]

6,18-Bis(diethoxycarbonylmethylidene)-1-phenyl-phenanporphodimethene (**1**-H) was synthesized by [2+2] acid condensation of the 1,10-phenathroline vinyl derivative **3** with the corresponding *meso*-phenyldipyrromethane[16] **4**. This synthetic route provides the advantage of preparing the desired compounds in one pot without forming a complex mixture (Scheme 1). Compound **3** was prepared by a Knoevenagel condensation of 1,10-phenanthroline carbaldehyde (**2**) with diethylmalonate to deliver **3** in a 72% yield. Compound **3** and the corresponding phenyldipyrromethane **4** were stirred at room temperature for 48 hours in the presence of trifluoroacetic acid (TFA). A subsequent oxidation by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) afforded the desired compound **1**-H in a 63% yield. The formation of **1**-H was confirmed by NMR and HRMS



Scheme 1. Synthesis of **1**-H.

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analyses, and elemental analysis. The ^{1}H NMR spectrum of **1**-H reveals a significant downfield shift of the pyrrole NH resonance to $\delta = 13.62$ ppm and the appearance of β-pyrrolic protons in the typical alkene proton range with two sets of doublets ($J = 4.3$ Hz) at $\delta = 6.66$ and 6.49 ppm. These results indicate that the full macrocyclic π conjugation is interrupted due to the stable exocyclic double bonds at the meso positions. These results are also supported by the ^{13}C NMR spectrum.

The X-ray crystallographic analysis of **1**-H[17] indicates that the macrocycle has a gable-type nonplanar structure, similar to that of 5,15-dialkylidene and diimino porphyrin analogues (Figure 1).[18] The dihedral angle between the 1,10-phenanthroline plane and the dipyrromethene plane is approximately 120°, and on the basis of the inter-nitrogen

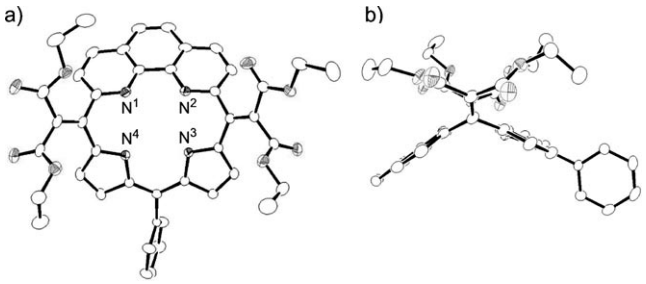

Figure 1. Molecular structure of **1**-H with thermal ellipsoids (50% probability). The hydrogen atoms and solvent molecules are omitted for clarity.; a) top view and b) side view.

distances, the nitrogen coordination environment has a smaller inner core than that of tetraphenylporphyrin (see Figure S1 and Table S1 in the Supporting Information).

When $MgCl_2$ is added to an acetonitrile solution of **1**-H under ambient conditions, the solution color changes from reddish-orange to purple. This change is coincident with a red-shift of the visible band from 489 to 532 and 565 nm with increased intensity (Figure 2a). These spectral changes are caused by the formation of the corresponding Mg^{2+} complex, **1**-MgCl. The binding of Mg^{2+} within the inner core is confirmed in the ^{1}H NMR spectrum, which indicates loss of the proton signal for the H on the inner nitrogen atom upon addition of Mg^{2+}; all other aromatic proton signals remain (see Figure S2 in the Supporting Information). The complex formation between **1**-H and Mg^{2+} was additionally evidenced by detection of the corresponding mass peak at $m/z =$ 759.2285 (Calcd 759.2305 for [**1**-Mg]$^+$) in the HR/FAB/MS spectrum. It is likely that **1**-MgCl has square pyramidal geometry with an axially coordinated chloride anion as a result of the addition of $MgCl_2$. Similar geometries of zinc(II) complexes with monoanionic porphyrinoid ligands prepared in a similar manner have been reported.[11d,19]

Compound **1**-H exhibits extremely weak fluorescence ($\lambda_{max} = 572$ nm, $\Phi_H = 0.003$) in its free base form. Upon complex formation with Mg^{2+}, a stronger red-shifted fluorescence emission ($\lambda_{max} = 639$ nm, $\Phi_{Mg} = 0.015$) is observed with a slight quenching of the original emission of **1**-H (Figure 2b). The Mg^{2+} ion induces an approximately 16-fold emission enhancement in the intensity ratio between 572 and 639 nm.

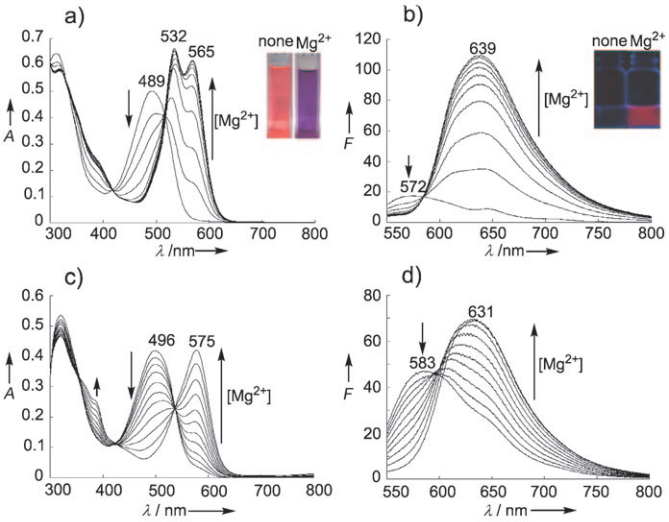

Figure 2. UV/Vis absorption (a) and fluorescent (b) spectral changes of **1**-H (2×10^{-5} M) upon titration with $MgCl_2$ in MeCN. , The isosbestic point in UV/Vis spectrum is at $\lambda_{ex} = 520$ nm. UV/Vis absorption (c) and fluorescent (d) spectral changes of **1**-H (2×10^{-5} M) upon titration with $MgCl_2$ in 0.1 M HEPES buffer (pH 7.4, KNO_3 ($I = 0.1$)) and DMSO solution (7:3 v/v). The isosbestic point in UV/Vis spectrum is at $\lambda_{ex} = 535$ nm. Insets of in a) and b) show the solution colors and fluorescent images ($\lambda_{ex} = 365$ nm) of **1**-H (left) and the magnesium complex (right).

This suggests that **1**-H is a good candidate for development of an efficient Mg^{2+}-responsive sensor for ratiometric measurements.[20] The ion selectivity of **1**-H was examined by titration of Na^+, K^+, and Ca^{2+}, which are physiologically important cations. Currently available fluorescent probes[21] for Mg^{2+} such as Mag-Fura-2, Magnesium Green, and others suffer from Ca^{2+} interference in the quantitative measurement of Mg^{2+} in cells. For the **1**-H system, emission enhancement was not induced by addition of Na^+ and K^+, and a limited fluorescent response for Ca^{2+} was observed. This data indicates that **1**-H has a lower affinity for Na^+, K^+, and Ca^{2+} relative to Mg^{2+} (Figure 3 and see Figure S3 in the Supporting Information). The binding constants (K_{Mg} and K_{Ca}) for the complex formation between **1**-H and Mg^{2+} and Ca^{2+} were estimated using nonlinear least-square analysis to be 4.4×10^6 and $7.3\times10^4\,M^{-1}$, respectively. Based on the association constant ratio (K_{Mg}/K_{Ca}), **1**-H exhibits selectivity for Mg^{2+} over Ca^{2+} as a result of the cation fitting[23] within the inner N4 core cavity (Ca^{2+}: 1.00 Å). Additionally, the biologically relevant d-block metal ions showed the spectral responses (quench or limited increase), these cations however would have little influence, since they exist at low concentrations[22] compared to Mg^{2+} (see Figure S4 in the Supporting Information). These results demonstrate that **1**-H could be an appropriate receptor for facile coordination and fluorescent detection of Mg^{2+} without major interference from Ca^{2+}.

To test the sensing ability of Mg^{2+} in an aqueous solution, **1**-H was treated with Mg^{2+} in 0.1M HEPES buffered aqueous DMSO solution (DMSO/H_2O = 7:3 v/v, pH 7.4). **1**-H exhibits a similar red-shift of the absorption band from 496 to 575 nm and of the emission band from 583 to 631 nm upon addition of

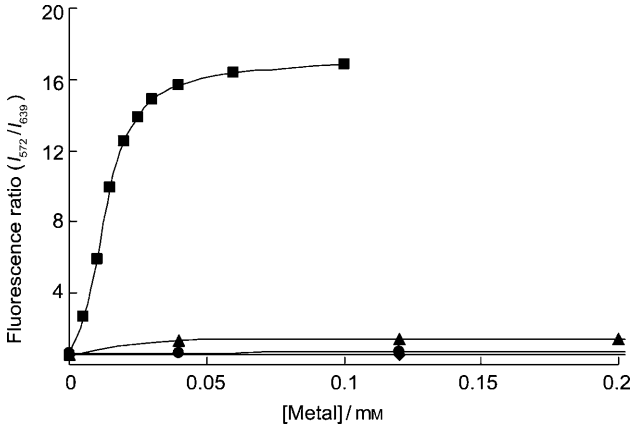

Figure 3. Plots of fluorescence intensity ratio between 572 and 639 nm (I_{572}/I_{639}) of **1**-H versus increasing concentration of various cations ($MgCl_2$: square, $CaCl_2$: triangle, NaCl: circle, and KCl: rhombus).

Mg^{2+} (Figure 2c and d). Generally, the complexation of Mg^{2+} by ligands in water is difficult to attain, because of the strong solvation of water molecules to Mg^{2+}. Accordingly, the ability of Mg^{2+} to form a complex with nitrogen ligands is low. However, **1**-H forms the **1**-MgCl complex even in the presence of water with a binding constant of $K_{Mg} = 37.3\,M^{-1}$. This corresponds to commonly observed intracellular free Mg^{2+} concentration levels (0.1–10 mM). The affinities of potentially interfering cations such as Ca^{2+} are lower than the affinity for Mg^{2+} under these conditions (see Figure S5 in the Supporting Information).

To gain additional understanding of the exited state character of **1**-H and **1**-MgCl, DFT calculations were performed at the B3LYP/6-31G(d) level of theory (Figure 4). The electronic densities of both the HOMO and the LUMO of the free base **1**-H, consisting of ligand-centered π orbitals, appear mainly at the dipyrromethene unit. This finding suggests that the dipyrromethene moiety functions as the fluorophore. In the optimized structure of **1**-MgCl, the Mg^{2+} has a square pyramidal geometry with an axially coordinated chloride ligand as expected (see Figure S6 in the Supporting Information). The density of the HOMO of **1**-MgCl has a similar distribution as that of the **1**-H, whereas the density of the LUMO of **1**-MgCl is delocalized over the whole macrocycle including the 1,10-phenanthroline unit. This property may provide stabilization of the LUMO energy level of **1**-MgCl relative to that of **1**-H (see Figure S7 in the Supporting Information). Moreover, it was found that no electron density exists on the magnesium center in the orbital diagrams of **1**-MgCl. It is postulated that the HOMO–LUMO transition corresponds to the emissive π–π* excited state as observed in bodipy (bodipy = 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene) derivatives.[24] Such localization of the electronic densities and increase of the structural rigidity of the macrocycle of **1**-MgCl upon Mg^{2+} complexation could be attributable to both the emission enhancement and red-shift.

In summary, we have successfully synthesized the novel porphyrin analogue **1**-H with an embedded 1,10-phenanthroline moiety. **1**-H has a gable-type nonplanar macrocyclic structure with a relatively small monoanionic coordination

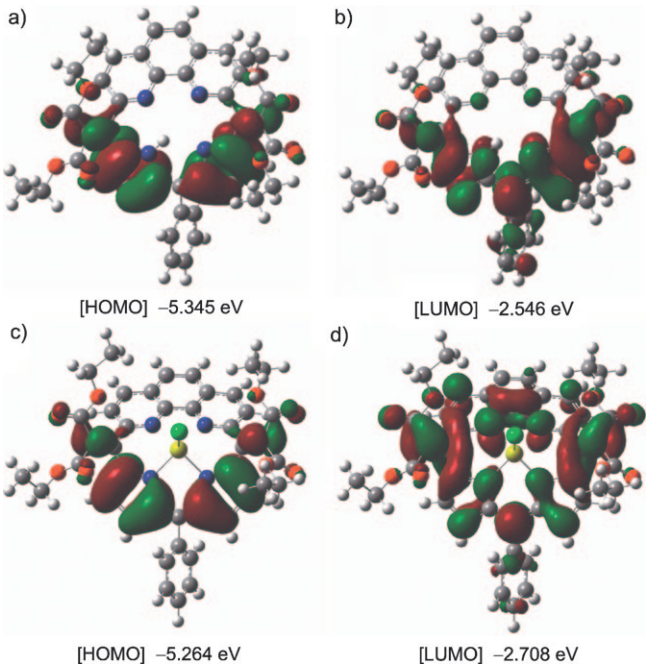

Figure 4. Molecular orbital plots of **1**-H and **1**-MgCl (B3LYP/6-31G(d)). a) HOMO and b) LUMO of **1**-H, c) HOMO and d) LUMO of **1**-MgCl.

sphere within the inner cavity. These interesting structural features of **1**-H enable facile Mg^{2+} complexation accompanied by significant emission enhancement and red-shift. These properties are consistent even in the presence of other physiologically important cations (Na^+, K^+, and Ca^{2+}). It is therefore expected that **1**-H could be exploited as a Mg^{2+}-responsive fluorescent sensor, which provides a ratiometric detection of Mg^{2+} by far-red fluorescent emission above 600 nm. Furthermore, to the best of our knowledge, **1**-MgCl is the first example of a Mg^{2+}–dipyrromethene complex, "MgDipy", being supported by the adjacent 1,10-phenanthroline moiety. As a preliminary result, **1**-H works as a fluorescent sensor for Mg^{2+} in a semi-aqueous solution and appears to be a promising prototype for a cell-permeable fluorescent sensor with the ability to assess the total content of cellular Mg^{2+}.

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[17] Crystallographic data for **1**-H: $C_{90}H_{80}O_{18}N_8$, Mw = 1561.66, triclinic, space group $P\bar{1}$, $a = 12.075(2)$, $b = 16.916(3)$, $c = 20.833(3)$ Å, $\alpha = 96.861(8)$, $\beta = 103.378(7)$, $\gamma = 107.234(7)°$, $V = 3872.2(11)$ Å^3, $Z = 2$, $D_{calcd} = 1.339\ g\,cm^{-3}$, $F(000) = 1640$, $R_1 = 0.045$ ($I > 2.0\sigma(I)$), ${}_wR_2 = 0.129$ (all data), GOF = 1.098. CCDC 749250 (**1**-H) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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