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Novel highly selective fluorescent chemosensors for Zn(II)

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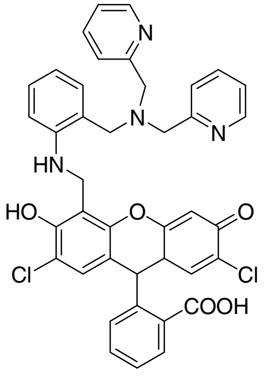
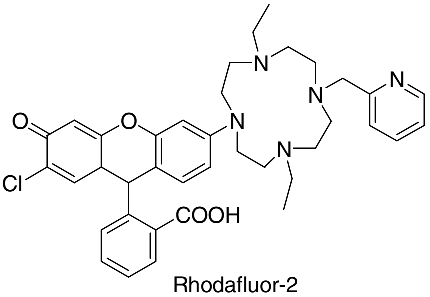
Abstract—Two novel fluorescent Zn^{2+} chemosensors were synthesized in four steps from inexpensive starting materials. They exhibited very strong fluorescence responses to Zn^{2+} and had remarkably high selectivity to Zn^{2+} than other metal ions including Mg^{2+}, Ca^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+}. These two new molecules could be used as low-priced yet high-quality Zn^{2+} chemosensors.

Zn^{2+} is the second most abundant transition metal cation in biology.[1] It is required for normal growth and development. It is also necessary for many cellular processes such as neurotransmission and apoptosis. Currently there is a great interest in the development of Zn^{2+} imaging tools for exploring the role of Zn^{2+} in medicine and biology. Toward this end, a number of Zn^{2+} sensor molecules such as Znpyrs, ZnAFs, and rhodafluors have been designed and synthesized.[2,3] The application of these Zn^{2+} chemosensors has led to many new exciting findings about the functions of Zn^{2+} in living systems.[4] Nonetheless, many of the currently available Zn^{2+} chemosensors are fairly complex molecules and their syntheses are often expensive. Furthermore, most of the currently available Zn^{2+} chemosensors still suffer from disadvantages such as high interference from other metal ions (in particular, Mg^{2+}, Ca^{2+}, Ni^{2+}, Cu^{2+}, and Cd^{2+}).[2]

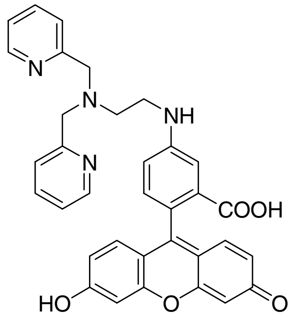
In an attempt to develop new types of Zn^{2+} chemosensors that are easier to synthesize, we paid attention to a very recent interesting finding by Urano et al. that the carboxylic group plays almost no role in the fluorescence properties of the fluorescein molecule.[5] Since fluorescence probes are excellent sensors and the removal of its carboxylic group will simplify its synthesis, we hypothesized that by attaching appropriate chelator groups to the decarboxylated fluorescein we would obtain some novel Zn^{2+} chemosensors. Herein we report the first successful examples of such chemosensors (**1** and **2** in Scheme 1) and their syntheses and characterizations.



Znpyr-4



Rhodafluor-2



ZnAF-1

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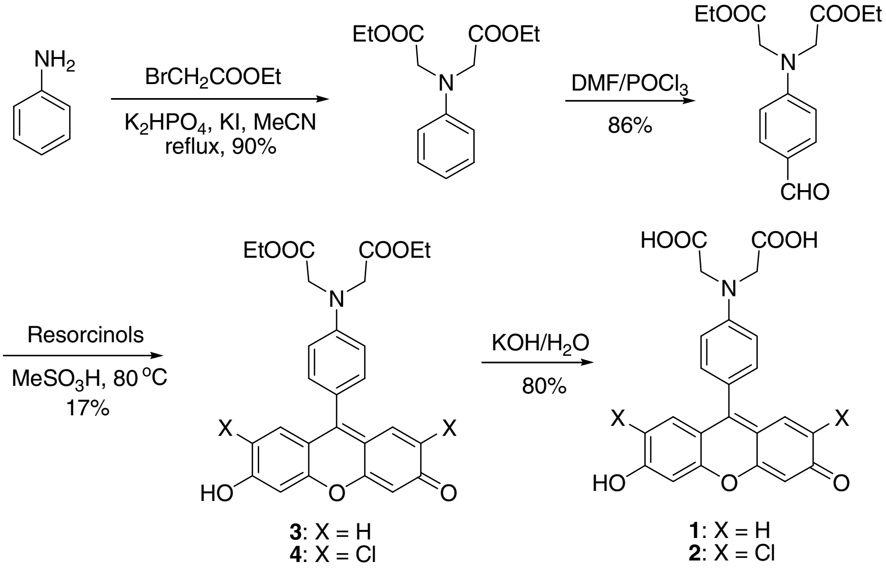
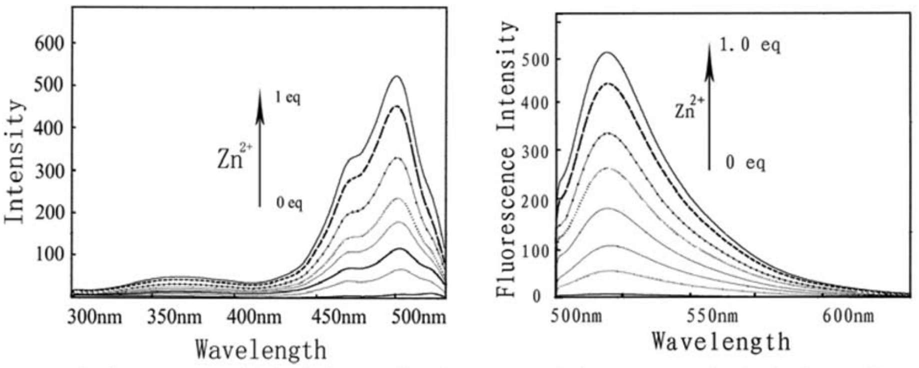


Scheme 1.

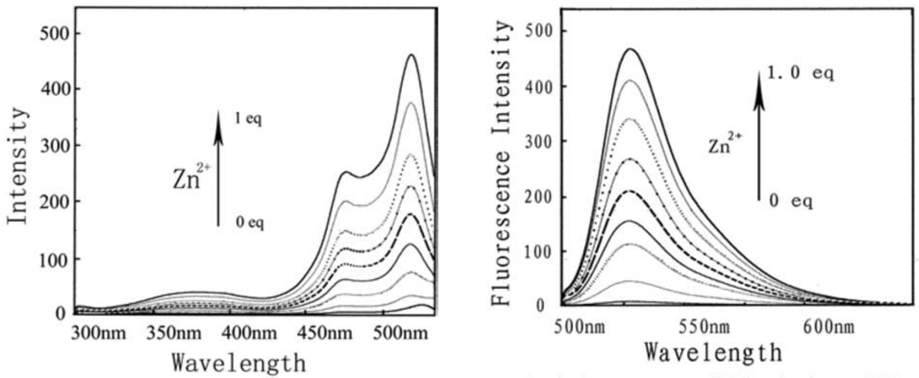
The syntheses of sensors **1** and **2** started from aniline (see Scheme 1). It was N-alkylated with ethyl bromoacetate (yield = 90%) and then formylated at the 4-position (yield = 86%) using the standard procedures. The subsequent acid-catalyzed condensation with resorcinol or 6-chlororesorcinol provided the desired decarboxylated fluoresceins (yield = 17%), which were hydrolyzed to give the final products (yield = 80%).[6–9] Thus, sensors **1** and **2** were successfully synthesized via four steps from readily available starting materials with an overall yield of 11%.

The maximum absorption wavelengths of **1** and **2** were 495 and 504 nm. The maximum emission wavelengths



Excitation spectrum of **1** (emission at 520 nm)

Emission spectrum of **1** (excitation at 488 nm)



Excitation spectrum of **2** (emission at 530 nm)

Emission spectrum of **2** (excitation at 495 nm)

Figure 1. The fluorescence responses of **1** and **2** upon the additions of Zn^{2+} (experimental conditions: 50 μM sensor, varying amounts of Zn^{2+}, 100 mM HEPES buffter, 0.1 M KCl, pH = 7.4).

of **1** and **2** were 520 and 534 nm. The fluorescence quantum yields of free **1** and **2** were 0.012 and 0.011 under physiological conditions (pH 7.4, 0.1 M KCl). Upon addition of Zn^{2+}, the fluorescence intensities of **1** and **2** increased by about 30-folds, and the corresponding quantum yields increased to 0.342 and 0.279, respectively (see Fig. 1). The mechanism for the fluorescence response was presumably the well-known photoinduced electron transfer (PET) mechanism.[10] It is important to point out that the 30-fold fluorescence enhancement of **1** and **2** is significantly higher than most of the previously reported Zn^{2+} sensors (which usually showed about 5–10-folds fluorescence enhancement).[2,3]

The dissociation constants, K_d, between the two new chemosensors were determined to be 7.8×10^{-8} and 4.1×10^{-8} M for **1** and **2**, respectively.[11] These two dissociation constants were comparable to the K_d values of some previously reported Zn^{2+} sensors (for instance, the K_d of FluoZin-3 was 1.5×10^{-8} M, and the K_d of RhodZin was 6.5×10^{-8} M).[2] The detecting limits of the two sensors were 2.0×10^{-8} and 5.0×10^{-8} M, respectively. Furthermore, a Hill plot analysis revealed that the maximum fluorescence could be obtained at 1:1 ratio, which suggested that **1** and **2** should both form a 1:1 complex with Zn^{2+}.

Having confirmed that **1** and **2** were fluorescent sensors for Zn^{2+}, we next paid attention to the selectivity of these two new sensors. Thus, we have studied the fluorescence responses of **1** and **2** to other metal cations including Cd^{2+}, Mg^{2+}, Ca^{2+}, Ba^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Mn^{2+} (see Fig. 2). It was found that **1** and **2** showed almost no fluorescence enhancement in the presence of Mg^{2+}, Ca^{2+}, Ba^{2+}, and Mn^{2+}. This is very nice because under many conditions (e.g., physiological conditions) Mg^{2+} and Ca^{2+} may exist at relatively high concentrations compared to Zn^{2+}.

It should be noted that the high selectivity of a Zn^{2+} sensor to Mg^{2+} and Ca^{2+} is actually not surprising, because the electronic structures of Mg^{2+} and Ca^{2+} are quite different from that of Zn^{2+}. On the other hand, because the electronic structures of Co^{2+}, Ni^{2+}, Cu^{2+}, and particularly Cd^{2+} are fairly similar to that of Zn^{2+}, most of the previous Zn^{2+} sensors do not exhibit good selectivity to these metal cations (for instance, the selectivity in many cases is close to 1:1 for Zn^{2+}:Cd^{2+}).[2] This may bring trouble to certain applications where Co^{2+}, Ni^{2+}, Cu^{2+}, or Cd^{2+} are interfering (e.g., in environmental science). Herein, **1** and **2** showed less than 4-fold fluorescence enhancement for Cd^{2+}, and less than 2-fold fluorescence enhancement for Co^{2+}, Ni^{2+}, and Cu^{2+} (see Fig. 2). Compared to the 30-fold fluorescence enhancement observed for Zn^{2+}, it was obvious that **1** and **2** were highly selective. This magnitude of selectivity was actually one of the best among all the known Zn^{2+} chemosensors. Therefore, **1** and **2** would be complementary to the

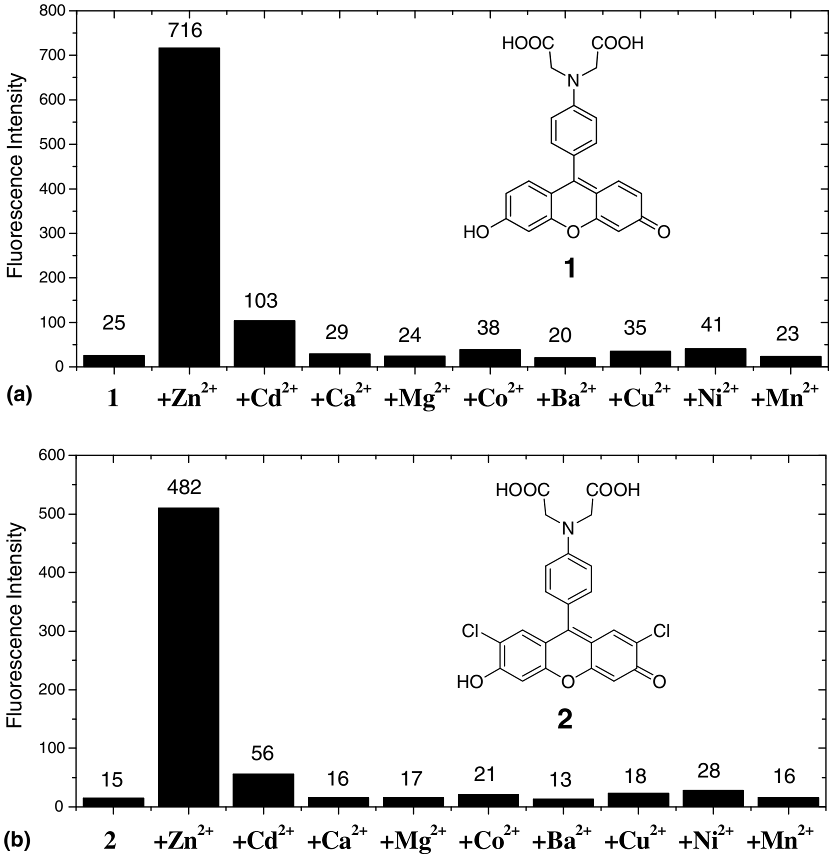


Figure 2. The fluorescence responses of **1** and **2** to different metal cations (experimental conditions: 20 μM sensor, 20 μM metal cation, 100 mM HEPES buffter, 100 mM KCl, pH = 7.4).

previous sensors in the applications where Co^{2+}, Ni^{2+}, Cu^{2+}, or Cd^{2+} coexists with Zn^{2+}.

In conclusion, we have designed, synthesized, and characterized two novel fluorescent chemosensors that are highly sensitive to Zn^{2+}. The synthesis of these two Zn^{2+} sensors was accomplished in four steps from inexpensive starting materials. The two new sensors showed much better selectivity to many other metal ions as compared to the previously reported Zn^{2+} sensors. Thus, the two new sensors reported here add novel tools to the arsenal of Zn^{2+} detection and imaging. Furthermore, the present study has confirmed Urano's very recent finding that the carboxylic group plays almost no role in the fluorescence properties of the fluorescein molecule.[5]

Acknowledgements

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6. Characterization of **3**: ^1H NMR (DMSO-d_6): δ 1.23 (6H, t, $J = 7.2$ Hz), 4.17 (4H, q, $J = 7.2$ Hz), 4.32 (4H, s), 6.58–6.66 (4H, m), 6.77 (2H, d, $J = 8.8$ Hz), 7.22 (2H, d, $J = 8.8$ Hz), 7.28 (1H, s), 7.31 (1H, s). ^{13}C NMR (DMSO-d_6): δ 14.3, 53.5, 61.4, 95.6, 104.8, 108.0, 110.2, 114.2, 117.4, 126.5, 128.5, 129.3, 130.1, 148.7, 149.5, 155.2, 157.3, 158.4, 159.6, 169.6, 185.8. HRMS (ESI): 476.1792 (calcd for $C_{27}H_{26}NO_7$ MH^+: 476.1748).
7. Characterization of **4**: ^1H NMR (DMSO-d_6): δ 1.21 (6H, t, $J = 7.2$ Hz), 4.15 (4H, q, $J = 7.2$ Hz), 4.33 (4H, s), 6.74–6.76 (2H, m), 6.83 (2H, d, $J = 8.5$ Hz), 7.28 (1H, s), 7.33 (2H, d, $J = 8.6$ Hz), 7.39 (1H, s). ^{13}C NMR (DMSO-d_6): δ 14.3, 53.3, 61.4, 96.7, 106.3, 109.5, 117.4, 119.3, 123.7, 127.3, 129.2, 130.0, 133.1, 148.8, 149.6, 155.6, 157.8, 159.1, 160.3, 169.7, 178.8. HRMS (ESI): 544.0935 (calcd for $C_{27}H_{24}Cl_2NO_7$ MH^+: 544.0927).
8. Characterizations for **1**: ^1H NMR (D_2O): δ 4.01 (4H, s), 6.27–6.32 (2H, m), 6.52 (2H, d, $J = 8.7$ Hz), 6.67–6.71 (2H, m), 7.06 (2H, d, $J = 8.7$ Hz), 7.25 (1H, s), 7.27 (1H, s). ^{13}C NMR (D_2O): δ 51.4, 104.3, 104.4, 110.3, 113.6, 113.6, 120.3, 123.3, 123.4, 133.5, 133.9, 134.7, 150.3, 157.9, 159.5, 169.3, 172.0, 181.4, 182.3. HRMS (ESI): 420.1089 (calcd for $C_{23}H_{18}NO_7$ MH^+: 420.1081).
9. Characterizations for **2**: ^1H NMR (D_2O): δ 4.07 (4H, s), 6.65–6.68 (2H, m), 6.70 (2H, d, $J = 8.4$ Hz), 7.28 (2H, d, $J = 8.5$ Hz), 7.40 (1H, s) 7.65 (1H, s). ^{13}C NMR (D_2O): δ 55.9, 104.3, 104.4, 111.4, 111.9, 112.8, 119.6, 127.8, 129.4, 129.8, 131.8, 131.9, 150.4, 157.0, 157.1, 157.3, 160.7, 174.2, 179.0. HRMS (ESI): 488.0309 (calcd for $C_{23}H_{16}Cl_2NO_7MH^+$: 488.0305).
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