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DPA-substituted coumarins as chemosensors for zinc(II): modulation of the chemosensory characteristics by variation of the position of the chelate on the coumarin

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The sensory capabilities of two novel di(2-picolyl)amine (DPA)-substituted coumarins are described and it is shown that the variation of the point of attachment of the DPA group to the coumarin framework controls their sensing behavior: the 4-substituted system is a CHEF-type sensor that shows a significant increase in fluorescence intensity upon Zn^{2+} binding, whereas the 3-substituted system is a ratiometric sensor.

The development of metal ion chemosensors in general, and for Zn^{2+} in particular, has received considerable attention.[1,2] The use of Zn^{2+}-specific chemosensors in biological systems promises to close the knowledge gap between the well-defined structural biochemistry of zinc and the understanding of zinc homeostasis and action.[3–7]

We recently reported a coumarin–cyclen-based chemosensor for Zn^{2+}.[5] Though this sensor proved capable of imaging Zn^{2+} in live cells, its binding kinetics was slow and the fluorescence intensity increase upon Zn^{2+} binding was a modest 4.4-fold. We report here the synthesis of coumarin–DPA-based chemosensors with much improved sensory characteristics, including the realization of a long-coveted ratiometric sensor for Zn^{2+}.[4a,7]

Nucleophilic substitution of 4-bromomethyl-6,7-dimethoxycoumarin (**1**) with DPA (**2**) produces sensor assembly **3** (Scheme 1).† DPA has proven its utility in the design of chemosensors for Zn^{2+}.[4] In addition, as an open chain chelate, it promised to alleviate the slow binding kinetics of our analogously constructed cyclen-based sensor.[5]

Fig. 1(A) shows the excellent chemosensory response of sensor **3**. Addition of 1 equiv. Zn^{2+} increases the integrated fluorescence intensity 23-fold. This chelation-enhanced fluorescence (CHEF) increase compares favorably to those of most known zinc-specific chemosensors.[6] The fluorescence quantum yields, ϕ, for **3** and **3**·Zn^{2+} in MeOH are 0.038 and 0.88, respectively (ε for **3** and **3**·Zn^{2+} in MeOH are 7600 and 6800 cm^{-1} M^{-1}, respectively). As expected, the binding of **3** to Zn^{2+} is fast and completed upon mixing of ligand and metal. Based on a Hill plot analysis, sensor **3** forms a 1:1 complex with Zn^{2+} and is suitable for measurements in aqueous media of pH 4–11 [Fig. 1(B)]. The K_d of the complex, measured by titration of **3** with Zn^{2+} in MeOH, is of the order of 0.5 μM. This value is in accord with those found for other DPA-based sensors.[4]

Relative fluorescence emission intensities ($I_{emission}$) observed in cells stained with a zinc-specific chemosensor can only be correlated with relative increases in [Zn^{2+}], but measurement of an absolute $I_{emission}$ does not allow the determination of an absolute [Zn^{2+}]. In part, this is because ϕ of any fluor is solvent dependent, but the solvent properties of the local environments in which the sensors accumulate are not known. The measurement of absolute [Zn^{2+}] can be achieved, however, with a ratiometric sensor.[8] A ratiometric probe responds upon binding to an analyte with a shift in its $\lambda^{max}_{emission}$. This shift should be large enough to allow the determination of the intensity ratio of the signals for co-existing Zn^{2+}-free and Zn^{2+}-bound species. Together with the known K_d of the sensor, this allows the determination of [Zn^{2+}].[8]

Ratiometric sensing behavior can be expected when the binding of the analyte changes the electronic properties of the chromophore, but the realization of this is non-trivial, as recent examples have shown.[4a,7] The lactone oxygen in coumarins is a potential donor atom attached to the chromophore, but steric restraints prevent the lactone oxygen in sensor **3** from participating in the coordination event. However, moving the attachment point for the chelating moiety on the coumarin from the 4- to the 3-position potentially allows for carbonyl participation. We therefore synthesized the sensor assembly **4** by reductive amination of coumarin aldehyde **5**[9] with DPA (Scheme 1).†

This particular 7-amino-derivatized coumarin derivative was chosen because it features longer $\lambda^{max}_{excitation}/\lambda^{max}_{emission}$ than 6,7-dime-

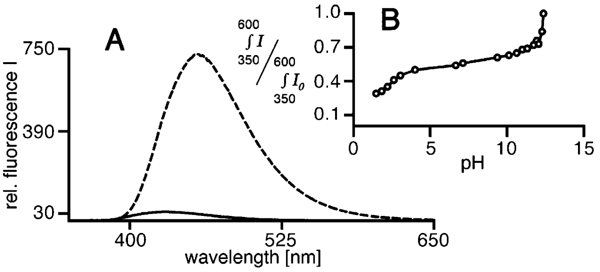
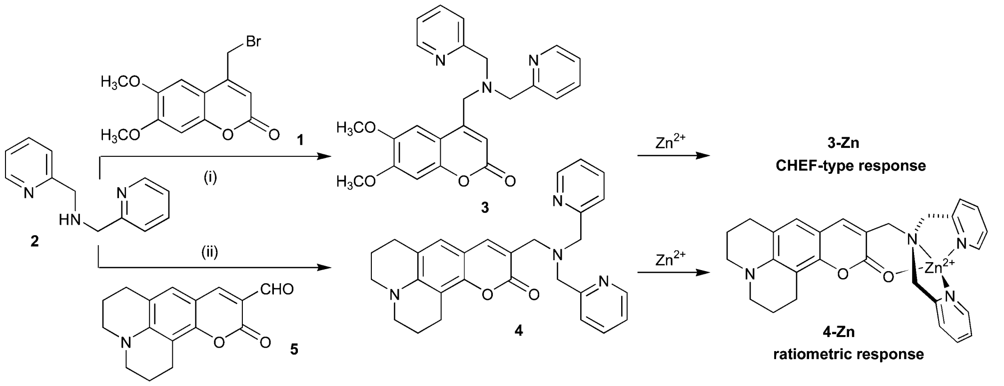


Fig. 1 (A) Emission spectra of **3** (—) and **3** + 1 equiv. Zn^{2+} (**---**). (B) pH-dependent fluorescence profile of **3**. Conditions: [**3**] = 192 μM in MeOH (A) and 10 μM in water containing 1 mM KOH and 100 mM KCl, pH adjusted with HCl (B); 25 °C; $\lambda_{excitation}$ = 343 nm.



Scheme 1 *Reagents and conditions*: (i) CH_2Cl_2, Na_2CO_3, r.t., 1 day (90% yield); (ii) $ClCH_2CH_2Cl$, $NaBH(OAc)_3$, r.t., 1 day (92% yield).†

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thoxycoumarin ($\lambda^{\max}_{\text{excitation}}$ = 400 nm for **4**, as compared to 343 nm for **3**). The use of longer wavelengths has a number of practical advantages in (confocal) fluorescence microscopy.

The results of a spectrophotometric titration of **4** with Zn^{2+} are shown in Fig. 2. The 31 nm shift of $\lambda^{\max}_{\text{absorption}}$ upon addition of Zn^{2+} demonstrates the Zn^{2+}-induced perturbation of the electronic structure of the chromophore. Thus, a ratiometric fluorescence response can be expected and, indeed, is observed. Incremental additions of Zn^{2+} result in a 21 nm bathochromic shift of $\lambda^{\max}_{\text{emission}}$ of **4**. This shift is solvent dependent and is minimized in solvent systems containing increasing amounts of water. We suggest the structure shown in Scheme 1 for **4**·Zn^{2+}. The fourth coordination site of the tetrahedrally N_3O-coordinated metal center is provided by the lactone oxygen, although the formation of a pentacoordinate metal center by inclusion of a water or alcohol molecule cannot be excluded.[3a] The solvent dependency of the $\lambda^{\max}_{\text{emission}}$ shift suggests that water is successfully competing with the carbonyl oxygen for coordination to the metal center, resulting in degradation of the ratiometric response.

In stark contrast to sensor **3**, **4** exhibits only minimal CHEF-type behavior. Sensor **4** in its free-base form is already 'switched on', with a ϕ of 0.64 (ε = 16 900 cm^{-1} M^{-1}). Thus, a methyleneamino group attached to the 3-position of the coumarin does not quench the fluorescence of free-base **4** effectively; therefore, chelation results only in a minimal increase in the fluorescence intensity. This, however, is not a disadvantage for the construction of ratiometric sensors.

The selectivity of sensors **3** and **4** for Zn^{2+} makes them suitable for use in biological systems. Fig. 3 shows the results of an M^{n+} binding and competition study of **3** (the profile of **4** is very similar to that of **3**). While a range of metals bind to the sensor, the addition of 1 equiv. Zn^{2+} outcompetes most. The paramagnetic ions Ni^{2+} and Cu^{2+} remain bound, but due to their fluorescence quenching properties, these ions will not provide a false positive signal mimicking the presence of Zn^{2+}. As is observed for most chemosensors for Zn^{2+}, Cd^{2+} binds strongly to the sensor. For sensor **4**, Cd^{2+} also elicits the same ratiometric response as Zn^{2+}. However, the concentration of Cd^{2+} in healthy cells is low. Thus, in practice, this ion will not interfere with the measurement of Zn^{2+} in live cells. The results of the biological evaluation of the sensors will be published in due course.

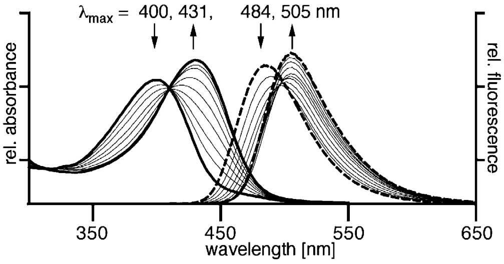

Fig. 2 (—) UV-vis spectral titration of **4** with Zn^{2+} (0–1 equiv.). (**---**) Fluorescence response upon titration of **4** with Zn^{2+} (0–1 equiv.); $\lambda_{\text{excitation}}$ = 410 nm. Conditions: [**4**] = 100 μM in MeOH; 25 °C.

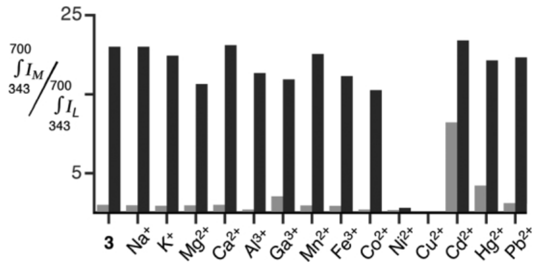

Fig. 3 M^{n+}-selectivity profile of sensor **3**: (grey bars) relative integrated emission intensity of **3** + 1 equiv. M^{n+}; (black bars) relative integrated emission intensity of **3** + M^{n+}, followed by 1 equiv. Zn^{2+}. Conditions: [**4**] = 163 μM in MeOH; $\lambda_{\text{excitation}}$ = 343 nm; 25 °C.

In conclusion, we have synthesized novel chemosensors for zinc(II) and have refined the design paradigms for coumarin-based CHEF-type and ratiometric sensors.

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Notes and references

† *Selected experimental data* for **3**: ^1H NMR ($CDCl_3$, 400 MHz): δ 8.54 (d, *J* = 4.1 Hz, 2H), 7.68–7.64 (m, 2H), 7.45 (d, *J* = 7.8 Hz, 2H), 7.31 (s, 1H), 7.20–7.17 (m, 2H), 6.82 (s, 1H), 6.60 (s, 1H), 3.94 (s, 3H), 3.90 (s, 9H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 161.8, 158.7, 153.2, 152.9, 149.9, 149.4, 146.2, 136.9, 123.4, 122.6, 112.4, 111.5, 106.0, 100.1, 60.9, 56.7, 56.5, 55.7 ppm; HR-MS (FAB+ of MH^+, NBA): *m/z* calcd for $C_{24}H_{24}N_3O_4$ 418.4744, found 418.4737. For **4**: ^1H NMR ($CDCl_3$, 400 MHz): δ 8.52 (d, *J* = 4.8 Hz, 2H), 7.71 (s, 1H), 7.66–7.64 (m, 4H), 7.15–7.11 (m, 2H), 6.87 (s, 1H), 3.92 (s, 4H), 3.66 (s, 2H), 3.25 (q, *J* = 5.5 Hz, 4H), 2.88 (t, *J* = 6.4 Hz, 2H), 2.76 (t, *J* = 6.4 Hz, 2H), 1.99–1.96 (m, 4H) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.8, 159.5, 151.0, 149.0, 145.4, 141.9, 136.4, 124.8, 122.8, 121.9, 118.2, 117.4, 108.5, 106.4, 60.1, 53.2, 49.9, 49.6, 27.5, 21.5, 20.6, 20.3 ppm; HR-MS (FAB+ of MH^+, NBA): *m/z* calcd for $C_{28}H_{29}N_4O_2$ 453.5666, found 453.5658.

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