Fluorescent Probe for Oxalic Acid: Design, Synthesis, and Evaluation

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A novel-type of structurally simple fluorescent chemosensor for oxalic acid was designed, synthesized, and evaluated to show that it detects oxalic acid in methanol with high selectivity over other anions including mono- and dibasic carboxylic acids.

Key Words : Chemosensor, Oxalic acid, Photoinduced electron transfer, Cyclen, Isothermal titration calorimetry

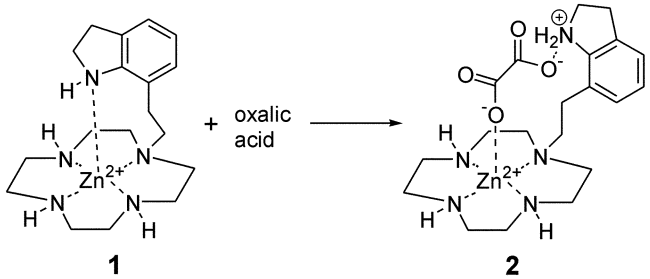
Introduction

Most of chemosensors are constructed by linking covalently a signaling unit to a receptor that recognizes and binds an analyte molecule.[1] The change in electron density surrounding the receptor molecule upon binding of an analyte to the receptor is transferred to the signaling unit by a number of ways, bringing about an optical signal.[1] Recently, Anslyn explored a new approach, in which the signaling unit is bound to a receptor noncovalently, and an analyte is allowed to compete for the receptor with the signaling unit. The successful displacement of the receptor-bound signaling unit by the analyte results in a chromogenic or fluorogenic modulation.[2,3] We wish to report herein the design, synthesis and evaluation of a novel probe that detects oxalic acid with a high selectivity. Oxalic acid is abundantly present in nature and widely used in industry. Ingestion of a large amount of food rich in oxalic acid can cause a loss of calcium in the blood and an injury to the kidney. An excess oxalic acid in the urine may be an indicative of renal failure, kidney lesions, and pancreatic insufficiency.[4] Accordingly, there have been reported numerous detection methods for oxalic acid.[5]

Zn^{2+} complex formed with 1,4,7,10-tetraazacyclododecane, *i.e.*, Zn^{2+}-cyclen is known to form complexes with certain anions such as thymidine.[6,7] It was thus thought that Zn^{2+}-cyclen can be of use as a binding site for one of the two carboxylates of the analyte of interest, *i.e.*, oxalic acid, and indoline that is linked to the cyclen by an alkyl chain was chosen as the binding site for the other carboxylate in the analyte as well as the signaling unit for the probe. The choice of indoline was based on the fact that it is sufficiently basic ($pK_a = 4.58$)[8] to undergo an ionic interaction with a carboxylate. Furthermore, indoline is known to exhibit fluorescence at 362 nm (in water) when excited at 288 nm.[9] It is not unreasonable to expect that the amino group in the indoline that is tethered to Zn^{2+}-cyclen (receptor) with an alkyl chain of appropriate length would form a coordinative bond to the metal ion in the receptor. However, since the coordination propensity of a carboxylate towards Zn^{2+}-cyclen is greater than that of the indoline amino group,[7] the Zn^{2+}-bound indoline would be displaced by a carboxylate upon the ternary complex of indoline, Zn^{2+}, and cyclen being exposed to oxalic acid, and the displaced indoline would engage in an ionic interaction with the other carboxylate of the receptor-bound oxalic acid to result in modulation of the fluorescence of indoline. In the system, the selectivity for a specific dicarboxylate may be achieved by adjusting the length of the alkyl chain that bridges the receptor and indoline. An ethylene chain was found to be most advantageous for the probe for oxalic acid. Lastly, in order for the probe to be functional, the polarity as well as solubilizing power of the medium is critical. We found that anhydrous methanol serves quite satisfactorily.[10] The design rationale is depicted schematically in Figure 1.

The probe was synthesized following the general route reported by Patinec *et al.*[11] as outlined in Scheme 1. Briefly, compound **3**[12] was condensed with molybdenumtricarbonyl-cyclen (**4**)[11] and the enamine product thus obtained was reduced *in situ* with sodium borohydride and subsequent air oxidation under acidic conditions removed the molybdenumtricarbonyl to afford **5**.

The fluorescence intensity of the uncomplexed ligand (**5**) in anhydrous methanol was diminished slightly upon the addition of zinc perchlorate, suggesting that the pendant indoline interacts with the Zn^{2+} in **1**. Although addition of malonic acid (10 μM) to the methanolic solution of **1** (10 μM) caused a slight decrease of the fluorescence intensity, succinic or glutaric acid (10 μM, respectively) rather



1 **2**

Figure 1. Design rationale for a chemosensor that detects oxalic acid selectively.

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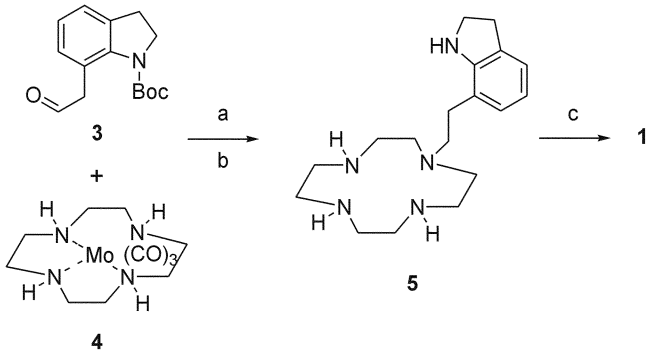

Scheme 1. Reagents and conditions: (a) **3**, **4**, DMF, excess $MgSO_4$, 100 °C, 6 h; (b) $NaBH_4$, rt, overnight; (c) $Zn(ClO_4)_2{\cdot}6H_2O$, MeOH, rt, 1 h.

increased the fluorescence intensity (Figure 2). Inorganic anions such as chloride, nitrate, sulfate, phosphate, and carbonate did not affect the fluorescence intensity of **1**. Oxalic acid did, however, decrease the fluorescence markedly as can be seen in Figure 2, and the decrease of fluorescence intensity is shown to be dependent on the concentration of oxalic acid up to the concentration equivalent to that of the probe as shown in Figure 3. Interestingly, addition of an equivalent amount of sodium oxalate to the methanolic solution of **1** caused only a slight attenuation of the fluorescence intensity. Addition of 2 equivalent amount of acetic acid caused rather an increase of the fluorescence intensity like the succinic and glutaric acids did (Figure 2). These observations put together suggest strongly that the fluorescence intensity attenuation caused by oxalic acid may not be due to the protonation of the amino group in the indoline by the oxalic acid but rather may possibly arise as a result of an electron transfer from the sensor-bound oxalic acid to the photo-excited indoline. In order to substantiate the proposition, we examined the binding mode of oxalic acid to **1** with the CPK model to learn that one of the oxygen atoms in the oxalic acid carboxylate that would undergo ionic interactions with the protonated amino group in the indoline rests closely above the indoline aromatic ring. We

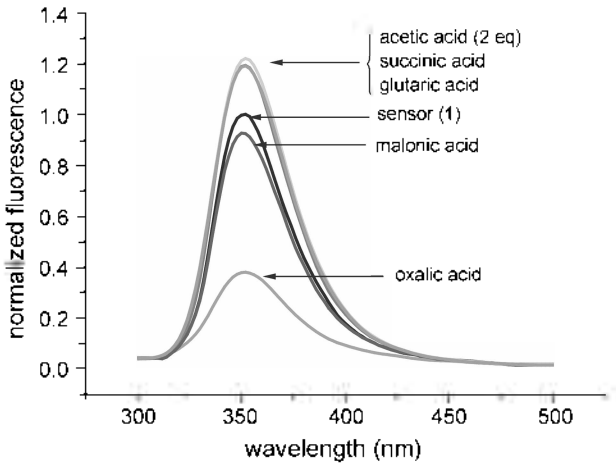

Figure 2. Fluorescence responses of **1** (10 μM) to various carboxylic acids (10 μM) in methanol. (λ_{ex} = 295 nm).

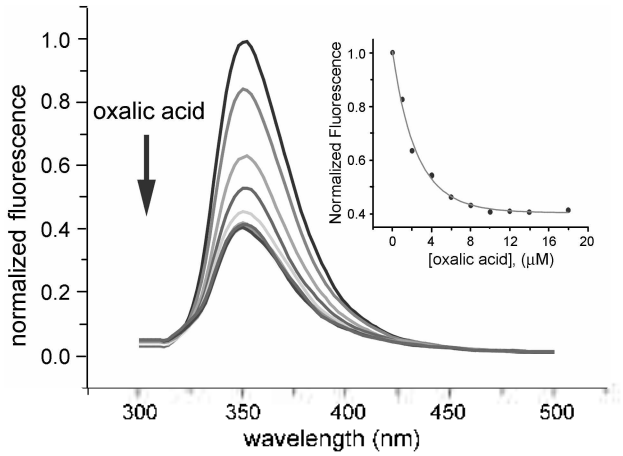

Figure 3. Fluorescence emission (λ_{ex} 295 nm) of **1** in methanol as a function of added oxalic acid. Inset: Spectrofluorometric titration of **1** (10 μM) in methanol with oxalic acid (λ_{ex} = 295 nm, λ_{em} = 350 nm).

are hence inclined to attribute the fluorescence quenching of **1** brought about by the oxalic acid to an electron transfer from the electron rich oxygen atom in the carboxylate to the HOMO of the photo-excited indoline aromatic ring (Figure 4). Such a phenomenon referred to photoinduced electron transfer (PET) has been well documented in the literature.[13,14]

The ^{1}H-NMR(MeOH-d_4) signal that appeared at δ 3.52 due to the protons at the 2-position of the indoline in **5** was shifted downfield by 0.20 ppm upon the addition of zinc perchlorate to yield **1**, which together with the fluorescence attenuation observed upon the addition of Zn^{2+} ion to the ligand **5** supports the proposition that metal-ligand interactions are in effect between the indoline amino group and the Zn^{2+} in **1**. The latter signal experiences a further downfield shift to δ 3.87 upon the addition of oxalic acid as expected from the design rationale.[15] The aromatic protons in **1** also experience analogous chemical shifts. We have performed isothermal titration calorimetry (ITC) experiments to obtain ΔH = -(6.17 ± 0.02) kcal mol^{-1}, ΔS = -1.71 eu, and K_{ass} = (1.18 ± 0.06) × 10^4 M^{-1} for the complex formation between **1** and oxalic acid in methanol (Figure 4). The

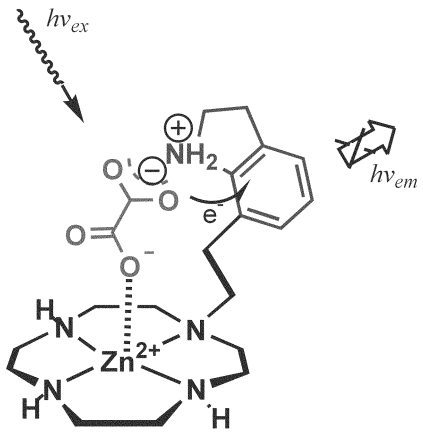

Figure 4. Schematic illustration for the fluorescent quenching effected by the binding of oxalic acid to **1**.

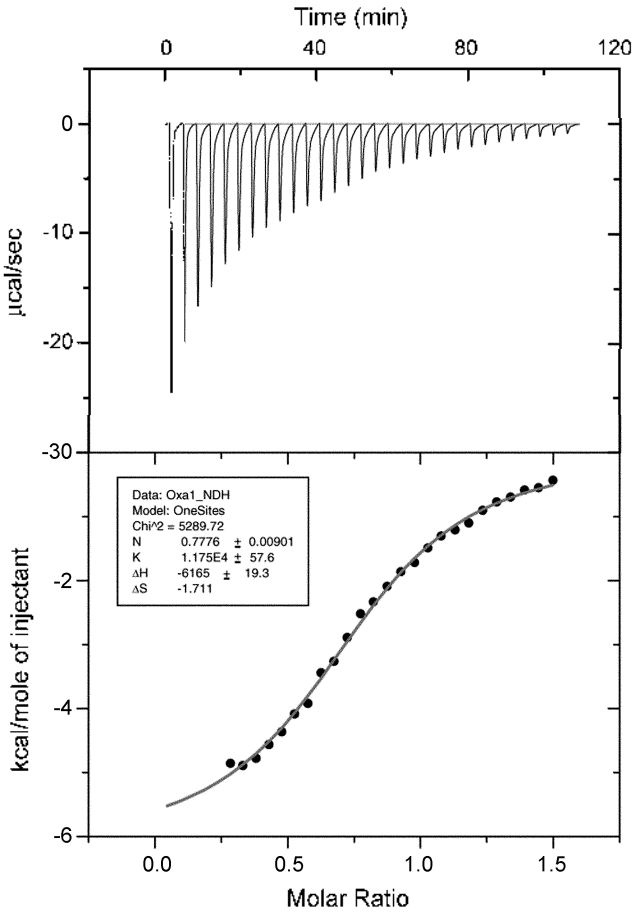

Figure 5. Isothermal calorimetric titration of **1** (1.0 mM) with oxalic acid (10 mM) in methanol at 30 °C. A methanolic solution (1.5 mL) of **1** (1.0 mM) was added to the calorimeter cell. To this solution was injected a 7 μL portion of the methanolic solution of oxalic acid (10 mM) 30 times. The mixture was continuously stirred and was kept at an operating temperature of 30 °C. The data were analyzed and fitted using the software Origin. Inset: Thermodynamic parameters (ΔH°, ΔG° and ΔS°), association constant (K_{ass}), and stoichiometry (n) for the binding of oxalic acid to **1** in methanol solution.

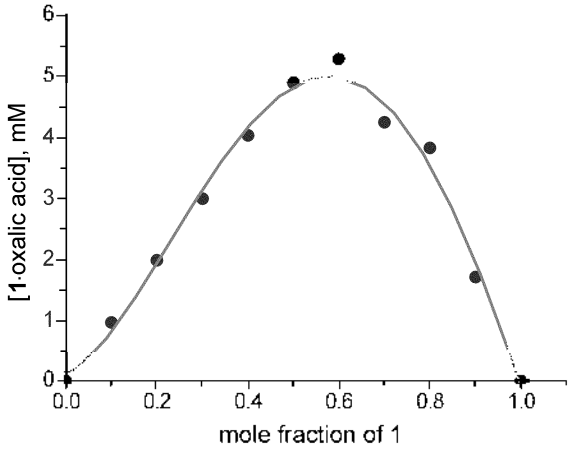

Figure 6. Job plot of sensor (**1**) with oxalic acid in methanol at 25 °C. Methanolic solutions of sensor (**1**) (10 mM) and oxalic acid (10 mM) were mixed in varying ratios and the chemical shifts of the protons at the 2-position of the indoline in the sensor were measured. The stoichiometry was obtained from the simple titration plot of [**1**·oxalic acid] vs mole fraction of **1**. In the figure, [**1**·oxalic acid] $\Delta\delta X_1[\mathbf{1}]_o$, in which $\Delta\delta$ $(\delta_{obs} - \delta_1)/(\delta_{1\cdot\text{oxalic acid}} - \delta_1)$, $X_1 = [\mathbf{1}]/([\mathbf{1}] + [\text{oxalic acid}])$, and $[\mathbf{1}]_o = 10$ mM.

thermodynamic parameters reveal that the binding is primarily enthalpy driven, overcoming the unfavorable entropy change associate with the binding of oxalic acid to **1** to form **2**.[16,17] The 1 : 1 stoichiometry suggested by the ITC method for the binding was confirmed by a Job plot[18] obtained with the ^{1}H-NMR data for the binding interactions (Figure 6).

In conclusion, compound **1** was designed and synthesized as a novel fluorescent chemosensor for oxalic acid. Whereas most of carboxylic acids increased the fluorescence intensity of **1** when tested in a methanolic solution, oxalic acid attenuated it markedly. The fluorescence quenching is thought to be resulted by an electron transfer from one of carboxylates in the sensor-bound oxalic acid to the HOMO of photoinduced exited indoline aromatic ring in **1**.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and were uncorrected. IR spectra were recorded on a Bruker Equinox 55 FT-IR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were obtained with a Bruker AM 300 (300 MHz) NMR spectrometer using tetramethylsilane as the internal standard. Low resolution mass spectra were obtained with a KRATOS MS 25 RFA instrument. High resolution mass spectra were obtained at Korea Basic Science Institute, Daejeon, Korea. Elemental analyses were performed at Center for Biofunctional Molecules, Pohang University of Science and Technology, Korea.

1-(2-(Indolin-7-yl)ethyl)-1,4,7,10-tertaazacyclododecane (5). To a solution obtained by dissolving **4**[10] (0.57 g, 1.64 mmol) in dry and degassed DMF (15 mL), an excess of dry $MgSO_4$ and **3** (0.39 g, 1.49 mmol) were added. The resulting mixture was heated under nitrogen atmosphere at 100 °C for 6 h. After cooling to room temperature, $NaBH_4$ (0.068 g, 1.79 mmol) was added and allowed to react overnight. The organic solvent was removed under reduced pressure. The residue was taken up in degassed 10% hydrochloric acid. The resulting acidic mixture (pH 1) was oxidized in air until no more carbon monooxide evolved, and then washed with CH_2Cl_2. The pH was raised to 14 with NaOH pellets in an ice bath. The solution was extracted with CH_2Cl_2 (25 mL × 3), and organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give an oil (0.24 g, 51%). The crude product thus obtained was purified by column chromatography as its fully *tert*-Boc protected form, then converted back into free **5**. To the crude product (0.24 g, 0.75 mmol) and triethylamine (0.47 mL, 3.40 mmol) in $CHCl_3$ (10 mL) was added a solution of di-*tert*-butyl dicarbonate (0.74 g, 3.40 mmol) in $CHCl_3$ (10 mL) at room temperature. The reaction mixture was stirred for 48 h at room temperature, and the organic solvent was removed under reduced pressure. The residue was purified by flash

column chromatography (silica gel 60, 230-400 mesh; eluant, hexane/EtOAc = 4/1) to give a solid, a derivative of **5**, in which all its amine moieties are protected with *tert*-Boc (0.22 g, 40%). Mp 94-95 °C; IR (KBr) 2975, 1689, 1456, 1366, 1248, 1162 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.45 (s, 27H), 1.50 (s, 9H), 2.78 (br, 6H), 2.95 (m, 4H), 3.32~3.52 (m, 12H), 4.03 (t, 2H) 7.03 (s, 3H); ^{13}C-NMR ($CDCl_3$) δ 26.29, 29.10, 29.18, 29.25, 29.40, 30.37, 47.79, 48.25, 48.67, 50.52, 51.60, 51.89, 53.25, 55.23, 78.12, 79.83, 80.04, 80.19, 81.28, 122.90, 125.52, 129.88, 130.82, 135.55, 142.10, 154.70, 156.07, 156.41, 156.79; Anal. Calcd. For $C_{38}H_{63}N_5O_8$: C, 63.57; H, 8.84; N, 9.75. Found: C, 63.36; H, 9.17; N, 9.55; (EI) m/z 717 (M^+).

To a solution of the fully protected **5** thus obtained (0.4 g, 0.28 mmol) in MeOH (10 mL) was introduced HCl gas at room temperature. The reaction mixture was stirred for 1 h and evaporated to give a white solid. (0.26 g) which was dissolved in water. The aqueous solution (10 mL) of the product (0.2 g, 0.43 mmol) was basified with 1 N NaOH solution, and then the solution was extracted with $CHCl_3$ (10 mL × 3). The combined organic layers were dried over anhydrous $MgSO_4$, and concentrated under reduced pressure to obtain the free ligand **5** as yellow oil (0.14 g, ~ 100%). IR (KBr) 3273, 2928, 2845, 1592, 1455, 1400 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.56 (m, 4H), 2.63 (s, 8H), 2.68 (m, 4H), 2.76 (m, 4H), 3.03 (t, 2H), 3.54 (t, 2H), 6.66 (t, 1H), 6.86 (d, 1H), 6.98 (d, 1H); ^{13}C-NMR ($CDCl_3$) δ 30.49, 30.81, 45.91, 46.48, 47.58, 47.75, 52.38 54.98, 119.53, 122.15, 123.09, 128.18, 129.86, 150.67; HRMS (FAB+) $(M+H)^+$: Cacld. For $C_{18}H_{32}N_5$, 318.2579. Found: 318.2660.

Zinc(II) Complex of 1-(2-(Indolin-7-yl)ethyl)-1,4,7,10-tertaazacyclododecane (1) To a methanolic solution (10 mL) of the free ligand (**5**) (0.1 g, 0.32 mmol) was added zinc perchlorate hexahydrate (0.12 g, 0.32 mmol), and the mixture was stirred for 1 h.

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References

1. (a) de Silver, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515. (b) Beer, P. D.; Schmitt, P. *Curr. Biol. Chem. Biol.* **1997**, *1*, 475. (c) Beer, P. D.; Gale, P. A. *Angew. Chem. Int. Ed.* **2001**, *40*, 486. (d) Martinez-Mánez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419.
2. Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963.
3. (a) Ait-Haddou, H.; Wiskur, S. L.; Lynch, V. M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 11296. (b) Wiskur, S. L.; Anslyn, E. V. *J. Am. Chem. Soc.* **2001**, *123*, 10109. (c) Metzger, A.; Anslyn, E. V. *Angew. Chem. Int. Ed.* **1998**, *37*, 649. (d) Niikura, K.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533. (e) Han, M. S.; Kim, D. H. *Angew. Chem. Int. Ed.* **2002**, *41*, 3809. (f) Fabbrizzi, L.; Marcotte, N.; Stomeo, F.; Taglietti, A. *Angew. Chem. Int. Ed.* **2002**, *41*, 3811.
4. Luque de Castro, M. D. *J. Pharmaceu. Biomed. Anal.* **1988**, *6*, 1.
5. (a) Cha, K. W.; Huang, H. Z.; Chai, H. C. *Bull. Korean Chem. Soc.* **2002**, *23*, 1456 and references cited thereof. (b) Sancenón, F.; Martinez-Mánez, R.; Miranda, M. A.; Segui, M.-J.; Soto, J. *Angew. Chem. Int. Ed.* **2003**, *42*, 647.
6. Shionoya, M.; Ikeda, T.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1994**, *116*, 3848.
7. Kimura, E. *Acc. Chem. Res.* **2001**, *34*, 171.
8. Albert, A.; Serjeant, E. P. *Ionization Constants of Acids and Bases*; Wiley & Sons: New York, 1962.
9. Allen, M. W.; Unruh, J. R.; Slaughter, B. D.; Pyszezynski, S. J.; Hellwig, T. R.; Kamerzell, T. J.; Johnson, C. K. *J. Phys. Chem. A* **2003**, *107*, 5660.
10. The probe fails to function in aqueous solution due to the high solvating power of water.
11. Patinec, V.; Yaouanc, J. J.; Clément, J. C.; Handel, H.; des Abbayes, H. *Tetrahedron Lett.* **1995**, *36*, 79.
12. (a) Andeerson, W. K.; Lai, G. *Synthesis* **1995**, 1287-1290. (b) Lai, G.; Anderson, W. K. *Tetrohedron* **2000**, *56*, 2583.
13. Fabbrizzi, L.; Faravelli, H.; Francese, G.; Licchelli, M.; Perotti, A.; Taglietti, A. *Chem. Commun.* **1998**, 971.
14. De Santis, G.; Fabbrizzi, L.; Licchelli, M.; Poggi, A.; Taglietti, A. *Angew. Chem. Int. Ed.* **1996**, *35*, 202.
15. The down field shifts of the proton resonance signals as well as the observed 1 : 1 stoichiometry in the binding of oxalic acid to **1** eliminates the possibility that the two carboxylates in the analyte bind the receptor Zn^{II} to form a complex of 1 : 2 stoichiometry.
16. The negative entropic contribution may be accounted for on the basis of the molecularity of the binding process, that is, two molecules binds to form one complex, and this entropy decrease surmounts the increase of entropy due to the release of solvated solvent molecules during the binding. It is well known that when a host-guest binding occurs, solvated solvent molecules are released into bulk solution, increasing the entropy of the overall system. See for example, (a) Meissner, R.; Garcias, S.; Mecozzi, S.; Rebek, Jr., J. *J. Am. Chem. Soc.* **1997**, *119*, 77. (b) Stödeman, M.; Dhar, N. *J. Chem. Soc. Faraday Trans.* **1998**, *94*, 899. (c) Prohens, R.; Rotger, M. C.; Pina, M. N.; Deyà, P. M.; Morey, J.; Ballester, P.; Costa, A. *Tetrahedron Lett.* **2001**, *42*, 4933.
17. We were unable to determine the association constant for the binding of free indoline to Zn^{II}-cyclen by the ITC method due to its poor ligating propensity to the Zn^{II}.
18. Conners, K. A. *Binding Constants, The Measurement of Molecular Complex Stability*; John Wiley and Sons: New York, 1987; pp 24-28.