



A thiourea-based chromoionophore for selective binding and sensing of acetate

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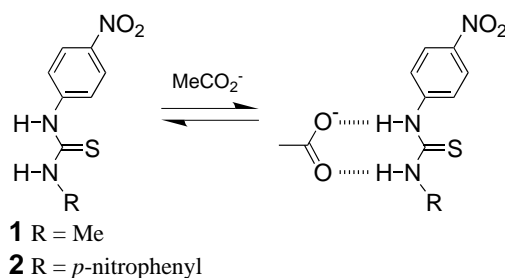
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Abstract—Highly selective binding and sensing of acetate over various monovalent inorganic anions ($\text{MeCO}_2^- \gg \text{H}_2\text{PO}_4^- > \text{Cl}^-$, Br^- , I^- , SCN^- , NO_3^- , HSO_4^- , ClO_4^-) are achieved by *N,N'*-bis(*p*-nitrophenyl)thiourea as a hydrogen-bonding chromoionophore in 1% water–99% MeCN (v/v), and acetic acid in vinegar is successfully determined by the complexation-induced chromogenic response of this chromoionophore. © 2001 Elsevier Science Ltd. All rights reserved.

The design and synthesis of hydrogen-bonding receptors for biologically and/or chemically important anions are of current interest in host–guest chemistry.^{1,2} Several applications of hydrogen-bonding receptors in transports and electrochemical sensors have been reported. Hydrogen-bonding receptors with redox-active and/or fluorogenic units have also been reported for selective anion detection. By contrast, despite their considerable advantage^{3–5} over other modes of signal transduction, there are only a few reports on chromogenic receptors that can recognize anions by a readily observable color change.^{2h,4,5}

We have recently shown that a very simple thiourea conjugated with one *p*-nitrophenyl unit (**1**) binds anions exclusively via formation of hydrogen bonds in MeCN and produces a color change with a selectivity of $\text{MeCO}_2^- > \text{H}_2\text{PO}_4^- > \text{Cl}^- \gg \text{ClO}_4^-$.^{6a} We have also shown that hydrogen bond-mediated complexation of anions in aqueous media can be achieved by **1** located deep inside the vesicle.^{6b} From a practical viewpoint, however, complex strengths, binding selectivity and optical responses are only moderate for **1** when used in the colorimetric sensing of anions. We now report on the synthesis of a thiourea-based chromoionophore with two *p*-nitrophenyl units (**2**) and show that both complex stability and optical responses can be significantly improved by another introduction of a *p*-nitrophenyl group into the (*p*-nitrophenyl)thiourea moiety. In con-

trast to many papers describing the complexation properties of various types of (thio)urea derivatives in 100% organic media,⁷ we investigated the complexation properties of **2** in *water-containing* medium rather than 100% organic medium. The experiment using water-containing medium is quite important to show applicability of synthetic molecules for the selective determination of ions normally exist in aqueous phase. Indeed, chromoionophore **2** is found to be more highly selective for acetate than various monovalent inorganic anions in 1% water–99% MeCN (v/v), making it possible to determine the concentration of acetic acid in vinegar.⁸



Compound **2** was synthesized analogously to **1** by reacting *p*-nitroaniline with *p*-nitrophenyl isothiocyanate in THF–triethylamine, and purified three times by recrystallization from THF.[†]

Keywords: hydrogen-bonding; ionophore; anion recognition; chromogenic sensing; thiourea.

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[†] Identifying data for **2**: ¹H NMR (270 MHz, in DMSO-*d*₆): δ 10.75 (s, br., 2H, NH), 8.23 (d, *J* = 9.2 Hz, 4H, *m*-ar), 7.83 (d, *J* = 9.2 Hz, 4H, *o*-ar). Anal. calcd for C₁₃H₁₀N₄O₄S (318.31): C, 49.05; H, 3.17; N, 17.6; S, 10.07%. Found: C, 49.04; H, 3.28; N, 17.55; S, 9.95%.

The effect of various anions as $N(C_4H_9)_4^+$ salt on absorption spectrum of **2** (2.0×10^{-5} M) was examined in 1% water–99% MeCN (v/v), and results are shown in Fig. 1. Spectrum (a) was measured in the absence of

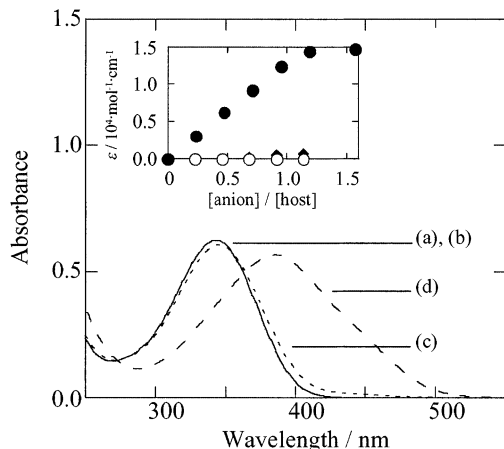


Figure 1. UV-vis spectra of **2** in 1% water–99% MeCN (v/v) (a) in the absence of anions and in the presence of (b) Cl^- , (c) $H_2PO_4^-$ and (d) $MeCO_2^-$. $[2]=[anion]=20 \mu M$. Cell length: 1.0 cm. Inset: dependence of molar extinction coefficient ϵ at 450 nm of **2** on the concentrations of (●) $MeCO_2^-$, (◆) $H_2PO_4^-$ and (○) other anions (Cl^- , Br^- , I^- , SCN^- , NO_3^- , HSO_4^- , ClO_4^-).

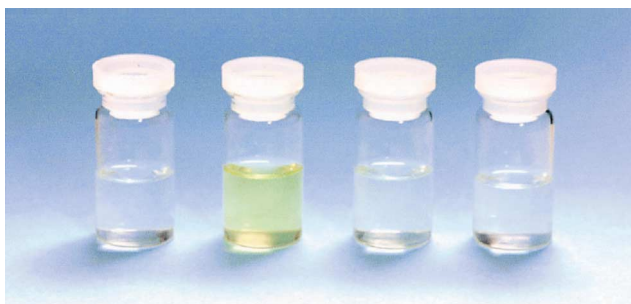


Figure 2. Effect of anions (as $N(C_4H_9)_4^+$ salt) on color changes of **2** in 1% water–99% MeCN (v/v). From left to right: **2**; **2**+ $MeCO_2^-$; **2**+ $H_2PO_4^-$; **2**+ Cl^- . $[2]=[anion]=20 \mu M$.

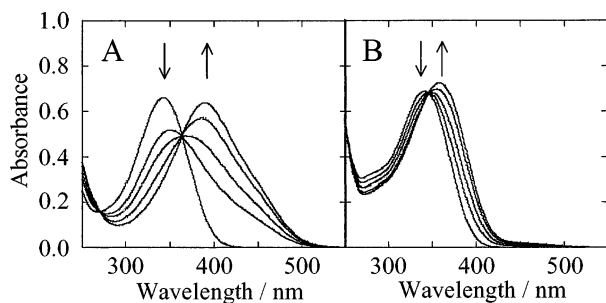


Figure 3. Changes in UV-vis spectra of **1** and **2** titrated with $MeCO_2^-$ (as $N(C_4H_9)_4^+$ salt) in 1% water–99% MeCN (v/v). (A) Chromoionophore **2** ($21 \mu M$) upon addition of $MeCO_2^-$ (0, 10, 15, 20, 30 μM). (B) Chromoionophore **1** ($50 \mu M$) upon addition of $MeCO_2^-$ (0, 50, 100, 200, 300 μM). Cell length: 1.0 cm.

anions, where **2** has a UV-vis spectrum with λ_{max} at 343 nm ($\epsilon=3.1 \times 10^4 M^{-1} cm^{-1}$), which can be assigned as an intramolecular charge transfer (CT) absorption band. As shown in spectrum (b), **2** exhibits negligible perturbation upon addition of 1 equiv. of Cl^- . Similarly, **2** does not show any obvious spectral change even in the presence of 1 equiv. of Br^- , I^- , SCN^- , NO_3^- , HSO_4^- and ClO_4^- , while a slight response is observed after addition of 1 equiv. of $H_2PO_4^-$ (spectrum c). By contrast, significant changes are observed in the presence of 1 equiv. of $MeCO_2^-$. As shown in spectrum (d), the CT absorption band appears at 392 nm with a shoulder absorption at around 450 nm, and the solution color changes from colorless to yellow (Fig. 2). The changes in molar extinction coefficient ϵ monitored at 450 nm as a function of anion concentration are illustrated in the inset of Fig. 1. It is apparent that **2** has higher selectivity for acetate than various monovalent inorganic anions under the examination conditions ($MeCO_2^- \gg H_2PO_4^- > Cl^-, Br^-, I^-, SCN^-, NO_3^-, HSO_4^-, ClO_4^-$).

The binding properties of **2** with $MeCO_2^-$ were further assessed by UV-vis spectroscopy. Fig. 3A shows the dependence of UV-vis spectra of **2** in 1% water–99% MeCN (v/v) on the concentration of $MeCO_2^-$. Increasing the concentration of $MeCO_2^-$ produces a significant bathochromic shift in the λ_{max} from 343 to 392 nm, and a clear isosbestic point appears at 364 nm. The changes in molar extinction coefficient ϵ at 450 nm as a function of the acetate concentration give a distinct titration endpoint at a 1:1 ratio of host and guest (cf. inset of Fig. 1). These observations are good evidence of 1:1 binding stoichiometry. In addition, a dilution of a 1:1 mixture of **2** and $MeCO_2^-$ in the binary solvent results in a decrease in ϵ at 450 nm, indicating that the acetate-induced change in the UV-vis spectrum can be ascribed not to proton transfer, but to complex formation through hydrogen bonding.⁹ The dilution experiment also allowed the determination of the stability of the acetate complex, which is $3.0 \times 10^5 M^{-1}$. Under identical conditions, a control chromoionophore **1** shows a much weaker response to acetate as shown in Fig. 3B (analysis: 358 nm; $K_{11}=5.6 \times 10^3 M^{-1}$; $\Delta\lambda_{max}=17$ nm). It is evident that another introduction of a *p*-nitrophenyl group into the (*p*-nitrophenyl)thiourea moiety enhances the hydrogen bonding ability, resulting in an extremely strong binding with acetate accompanied by more significant changes in the UV-vis spectrum. It is interesting to note that in MeCN the stability of the acetate complex of neutral thiourea-based receptor **2** ($K_{11}=1.7 \times 10^6 M^{-1}$) is going to reach that of the positively charged thiuronium-based receptor ($K_{11} > 10^6 M^{-1}$),^{2c} in which the favorable electrostatic attraction between the binding site and acetate, in addition to the hydrogen bonding interaction, should have considerable influence on anion binding events.^{2c,10} However, the difference in the substituting groups between these compounds should be mentioned for the comparison of binding constants. It is well demonstrated that phenyl substituted thiourea compounds such as chromoionophore **2** exhibit strong binding ability as compared with that of alkyl substituted ones.^{7a} All the thiuronium-based receptors

reported by Yeo et al.¹⁰ and Kubo et al.^{2c} are alkyl substituted compounds. Thus, it seems likely that hydrogen-bonding interaction between the reported thiuronium compound and anion is much weaker than that between our compound and anion, although electrostatic interaction is strong for thiuronium group.

Chromoionophore **2** was then applied to the colorimetric determination of acetic acid in a commercially available brand of vinegar. The analysis was carried out in 1% water–99% MeCN (v/v) by a standard addition method using the complexation-induced absorption change at 450 nm.[‡] The acetic acid concentration in the vinegar sample was determined to be 4.14±0.06 g/100 mL, which agrees with the specification provided by the supplier (4.2 g/100 mL). As compared with standard enzymatic methods that require use of three enzymes,^{8b} use of hydrogen-bonding chromoionophores^{8d} is a much simpler method to detect acetate (or acetic acid) in solution.

In summary, we have shown that highly selective, colorimetric sensing of acetate can be achieved by **2** in a binary mixture of water and MeCN. Although further improvements of the acetate selectivity over higher charged inorganic anions as well as other carboxylates may be necessary for the analysis of acetate in other types of samples, the concentration of acetic acid in the vinegar was successfully determined with **2**. Due to its very strong ability to form complexes with anions that act as hydrogen bond acceptors, receptor **2** might also be a promising candidate for various applications such as anion transports and/or electrochemical sensors,^{1,8c} which are now underway in our laboratory.

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[‡] Sample preparation: To eliminate interfering cations, 0.1 mL of vinegar was first passed through a cation exchange column (DOWEX-50-X8, H⁺-form) with deionized water as an eluting solvent. The pH of the eluent collected was adjusted to ca. 6.0 with N(C₄H₉)₄OH, followed by making up the volume to 100 mL with deionized water. An aliquot (0.1 mL) out of 100 mL was then diluted to 10 mL with MeCN containing **2** (20 μM) and MeCO₂⁻ (as N(C₄H₉)₄⁺ salt, 0–10 μM).

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