บทความวิจัย

ตัวตรวจจับไอออนลบโดย *N*,*N*-(*m*-xylylene)-bis-[*N*-(*p*-nitrophenyl)]dithiourea

แพน ทองเรือง* อัจฉราวดี แผนสนิท
วรรณนภา ย้วนบุญหลิม และ สมพร ติยะศรี

บทคัดย่อ

การสังเคราะห์ chromogenic anion sensor เพื่อตรวจจับไอออนลบโดยการเปลี่ยนสีทำได้อย่างง่ายและไม่ซับซ้อน การสังเคราะห์ thiourea-based receptor **1** ทำได้โดยการทำปฏิกิริยา coupling ระหว่าง *m*-xylylenediamine และ *p*-nitrophenylisothiocyanate ซึ่งให้ผลิตภัณฑ์ที่สูง การวิจัยนี้ได้ทำการพิสูจน์โครงสร้างของสารโดยใช้เทคนิค ^{1}H-NMR และ Mass spectroscopy และการวิเคราะห์ธาตุองค์ประกอบ ศึกษาการจับไอออนลบของ **1** โดยใช้เทคนิค ^{1}H-NMR titration และ UV-Vis Spectroscopy ผลที่ได้พบว่าค่าคงที่การเกิดสารประกอบเชิงซ้อนของ **1** กับ $H_2PO_4^-$ มีค่าสูงที่สุดเมื่อเปรียบเทียบกับไอออนลบตัวอื่นๆ ($H_2PO_4^- >> Cl^- >>> Br^-$, I^-, NO_3^- และ HSO_4^-) ความจำเพาะเจาะจงในการเลือกจับไอออนลบ สามารถอธิบายได้ในด้านรูปร่างสารประกอบเชิงซ้อน และความเป็นเบสของไอออนลบ Job's plot แสดงอัตราส่วนในการเกิดสารประกอบเชิงซ้อนของ **1** กับไอออนลบเป็น 1: 1 โดยการเกิดพันธะไฮโดรเจน การเปลี่ยนสีจากสีเหลืองใสเป็นสีส้มเมื่อมีการเติมไอออนลบฟลูออไรด์ในสารละลายของ **1** ใน DMSO ซึ่งสามารถใช้เป็นเซ็นเซอร์ที่มองเห็นด้วยตาเปล่าสำหรับไอออนลบฟลูออไรด์ได้

คำสำคัญ: ตัวตรวจจับไอออนลบ พันธะไฮโดรเจน ^{1}H-NMR titration thiourea-based receptor

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ภาควิชาเคมี คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ
*ผู้นิพนธ์ประสานงาน, email: pan@swu.ac.th

Anion Sensing by *N,N*-(*m*-xylylene)-bis-[*N*-(*p*-nitrophenyl)]dithiourea

Pan Tongraung*, **Acharavadee Pansanit**,
Wannapa Yuanboonlim and Somporn Tiyasri

ABSTRACT

A facile method for the synthesis of thiourea-based receptor chromogenic anion sensor **1** via coupling reaction of *m*-xylylenediamine and *p*-nitrophenylisothiocyanate was accomplished with high yield. The receptor was characterized by ^{1}H-NMR and mass spectroscopy and elemental analysis. The binding properties for various anions of **1** were examined by ^{1}H-NMR titration and UV-Vis Spectroscopic methods. The association constant of **1** with $H_2PO_4^-$ was the highest as compared to other anions ($H_2PO_4^- >> Cl^- >>> Br^-$, I^-, NO_3^- and HSO_4^-). The binding selectivity was explained in terms of the complex geometry and the basicity of the guest anions. Job's plot indicated that a 1: 1 stoichiometry complex was formed between **1** and anions through hydrogen bonding interactions. In particular, a distinct color change was observed from light yellow to orange upon addition of fluoride anion to the solution of **1** in DMSO. The receptor **1** can be applied as a naked-eye sensor for fluoride anion.

Keywords: chromogenic anion sensor, hydrogen bonding, ^{1}H-NMR titration, thiourea-based receptor

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Department of chemistry, Faculty of Science, Srinakharinwirot University
*Corresponding author, email: pan@swu.ac.th

Introduction

The design and synthesis of hydrogen-bonding receptors for biologically and chemically important anions are of current interest in host-guest chemistry [1, 2]. Synthetic anion receptors are generally composed of binding sites and co-valently linked signaling subunit. Anion binding sites include not only positively charged guanidinium or ammonium based on electrostatic interactions, but also neutral hydrogen-bonding donor group, such as urea or thiourea [3] and amides [4] by formation of hydrogen bonds. The development of chromogenic anion sensing is particularly challenging since visual detection can give immediate qualitative information and is becoming increasingly appreciated in terms of quantitative analysis. Most of these sensors have the chromophore covalently attached to the anion recognition unit [5, 6].

Chromogenic sensors for anions generally consist of two parts: anion receptors and chromophores. The concept of chromogenic anion sensors is illustrated in Figure 1. The ball can be compared to analytes such as anions and the cup acts like a receptor that is connected to a light bulb. When the ball goes into the cup that fits its shape, the electronic part of the light bulb is perturbed, and the light bulb will give a different color [7].

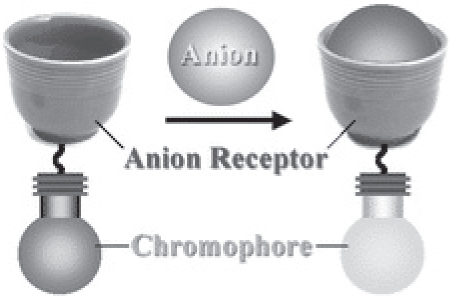

Figure 1 The concept of chromogenic anion sensors.

Anion recognition and sensor motifs are often structurally complicated and sophisticated synthetic process [8, 9]. Therefore, the investigation of a simple method for the synthesis of chromogenic sensors for anions was developed in this research, though there were previous reports of the development of easy-to-make chromogenic sensors using thiourea and calix[4]pyrole-chloranil [10, 11]. In this work we designed and synthesized chromogenic anion sensor 1 (Figure 2) by employing thiourea which was connected by p-nitrophenyl group as a chromophore, and reported binding properties of the receptor 1 with various anions using ^{1}H-NMR titrations and UV-Vis Spectroscopy methods. Anions such as $H_2PO_4^-$, HSO_4^-, NO_3^-, F^-, Cl^-, Br^- and I^- were chosen for this study because of their importance in environmental and biological systems. Results showed that both complex stability and optical response could be significantly improved by introduction of a *p*-nitrophenyl into the thiourea moiety, as a powerful naked-eye sensor for selective detection of F^- in DMSO.

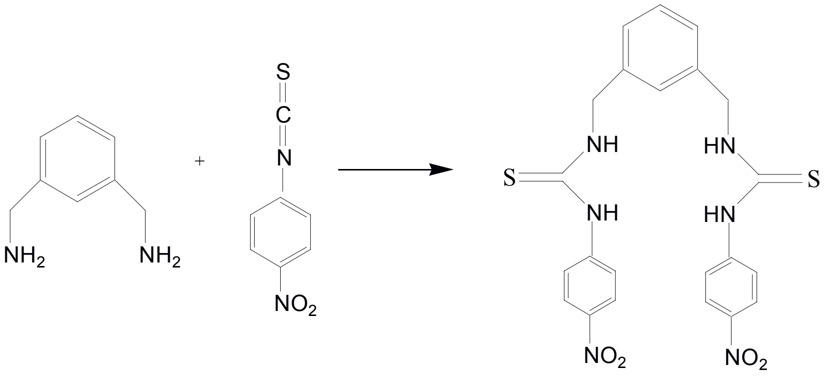

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Figure 2 Synthesis of chromogenic anion sensor 1.

Materials and Methods

m-Xylylenediamine, *p*-nitrophenylisothiocyanate and all guest anions in the form of tetrabutylammonium salts were purchased from Acros, USA, Aldrich, USA and Fluka, Switzerland. ^{1}H-NMR spectra were recorded on AVANCE 300 MHz Bruker. All chemical shift values (δ) were reported in parts per million (ppm), using the residual solvent signal (2.49) of DMSO-d_6 as reference. Absorption spectra were recorded by UV-2501PC spectrophotometer. Mass spectrum (MS-ESI) was determined on a Finnigan LC-Q. Elemental analysis was carried out on a Perkin-Elmer CHNON/ analyzer (PE 2400 series).

Synthesis of *N,N*-(*m*-xylylene)-bis-[*N*-(*p*-nitrophenyl)]dithiourea (1)

m-Xylylenediamine (0.20 g, 1.47 mmol) was dissolved in CH_2Cl_2 10 mL and a solution of *p*-nitrophenylisothiocyanate (0.58 g, 3.23 mmol) in CH_2Cl_2 10 mL was added slowly. The reaction solution was stirred under nitrogen atmosphere for 24 hours. A yellow solid was precipitated from the solution. The product 1 (0.58 g, 80%) was filtered and washed with CH_2Cl_2. ^{1}H-NMR (DMSO-d_6) δ 10.24 (br, 2H, NO_2ArNH-), 8.71 (br, 2H, $ArCH_2N\mathbf{H}$-), 8.16 (d, 4H, $NO_2ArHN\mathbf{H}$-, J = 9.0 Hz), 7.84 (d, 4H, $NO_2Ar\mathbf{H}NH$-, J = 9.0 Hz), 7.25-7.37 (m, 4H, $Ar\mathbf{H}CH_2NH$-), 4.77 (s, 4H, $ArC\mathbf{H}_2NH$-); ESI MS (m/z) : 495.0 [M^+]. Anal. Calcd for **1** ($C_{22}H_{20}O_4N_6S_2$): C, 53.22; H, 4.06; N, 16.94. Found : C, 53.29; H, 4.08; N, 16.96.

Complexation Studies of Ligand 1 with Anions (Cl^-, Br^-, I^-, NO_3^-, HSO_4^- and $H_2PO_4^-$) by ^{1}H-NMR Titration

A solution of 0.01 M of ligand **1** (5 x 10^{-6} mol) in DMSO-d_6 (0.5 mL) was prepared in a NMR tube. A series of solution of 0.10 M of tetrabutylammonium salts (5 x 10^{-5} mol, Bu_4NCl, Bu_4NBr, Bu_4NI, Bu_4NNO_3 Bu_4NHSO_4 and $Bu_4NH_2PO_4$) in DMSO-d_6 (0.50 mL) were prepared in a vial. The solution of anion was added directly to the NMR tube by micro syringe to give anion: ligand ratios as shown in Table 1 ^{1}H NMR spectra were recorded after each addition.

Table 1 Ratios of anion: ligand in DMSO-d_6.

Ratios of anion: ligand	Volume anion added (mL)	Mole anion in NMR tube (mol)	Total volume in NMR tube (mL)
0.0: 1.0	0.000	0.0	0.500
0.2: 1.0	0.010	1.0 x 10^{-6}	0.510
0.4: 1.0	0.010	2.0 x 10^{-6}	0.520
0.6: 1.0	0.010	3.0 x 10^{-6}	0.530
0.8: 1.0	0.010	4.0 x 10^{-6}	0.540
1.0: 1.0	0.010	5.0 x 10^{-6}	0.550
1.2: 1.0	0.010	6.0 x 10^{-6}	0.560
1.4: 1.0	0.010	7.0 x 10^{-6}	0.570
1.6: 1.0	0.010	8.0 x 10^{-6}	0.580
1.8: 1.0	0.010	9.0 x 10^{-6}	0.590
2.0: 1.0	0.010	10.0 x 10^{-6}	0.600

Complexation Studies of Ligand 1 with Anions (Cl^-, Br^-, I^-, NO_3^-, HSO_4^- and $H_2PO_4^-$) by UV-Vis Spectroscopy

A stock solution of 3 x 10^{-5} M of ligand 1 in DMSO was prepared by adding 2.5 mL of a stock solution (3 x 10^{-4} M) of ligand 1 to a 25 mL volumetric flask. The stock solution of ligand 1 (6 x 10^{-5} mol, 2 mL) was added to a 1 cm quartz cuvette. Absorption spectra of ligand 1 were recorded from 300 nm to 600 nm at ambient temperature. A solution of 1.5 x 10^{-3} M of tetrabutylammonium salt eg. (1.5 x 10^{-5} mol, Bu_4NF, Bu_4NCl, Bu_4NBr, Bu_4NI, Bu_4NNO_3 Bu_4NHSO_4 and $Bu_4NH_2PO_4$) in DMSO (10 mL) was prepared in a volumetric flask. The solution of a guest was added directly to the cuvette by a microburete and stirred for 30 seconds. Absorption spectra were measured after each addition to produce anion: ligand ratios as shown in Table 2.

Table 2 Ratios of anion: ligand in DMSO for complexation studies of ligands 1 with anions by UV-Vis Spectroscopy.

anion: ligand 1.5 x 10^{-3} M: 3 x 10^{-5} M	Ratios of anion: ligand	Volume of anion: Volume of 1 (mL)
No anion: 1	-	0: 2
Br^-: 1	50: 1	2: 2
Cl^-: 1	50: 1	2: 2
F^-: 1	50: 1	2: 2
$H_2PO_4^-$: 1	50: 1	2: 2
HSO_4^-: 1	50: 1	2: 2
I^-: 1	50: 1	2: 2
NO_3^-: 1	50: 1	2: 2

Color Change Tests of Ligands 1 with Anions (F^-, Cl^-, Br^-, I^-, NO_3^-, HSO_4^- and $H_2PO_4^-$)

The stock solution of ligand 1 (3 x 10^{-5} mol, 1 mL) was added to a vial. A solution of 3 x 10^{-5} M of tetrabutylammonium salt eg. (1.5 x 10^{-4} mol, 5 mL, Bu_4NF, Bu_4NCl, Bu_4NBr, Bu_4NI, Bu_4NNO_3 Bu_4NHSO_4 and $Bu_4NH_2PO_4$) in DMSO was added to the solution of 1 in vial, to give anion: ligand ratios as shown in Table 3. The solution of complexes was recorded to photograph.

Table 3 Ratios of anion: ligand in DMSO for color change tests of ligands **1** with anions.

anion: ligand **3×10^{-5} M: 3×10^{-5} M**	**Ratios of anion: ligand**	**Volume of anion: Volume of 1 (mL)**
No anion: **1**	-	0: 6
Br^-: **1**	5: 1	5: 1
Cl^-: **1**	5: 1	5: 1
F^-: **1**	5: 1	5: 1
$H_2PO_4^-$: **1**	5: 1	5: 1
HSO_4^-: **1**	5: 1	5: 1
I^-: **1**	5: 1	5: 1
NO_3^-: **1**	5: 1	5: 1

Results and Discussion

The anion sensor **1** shown in Figure 2 was readily synthesized by the coupling reaction between *m*-xylylenediamine and *p*-nitrophenylisothiocyanate in CH_2Cl_2. The compound was characterized by ^1H-NMR data, mass spectrometry and elemental analysis. Analytical data were consistent with the structure of **1**.

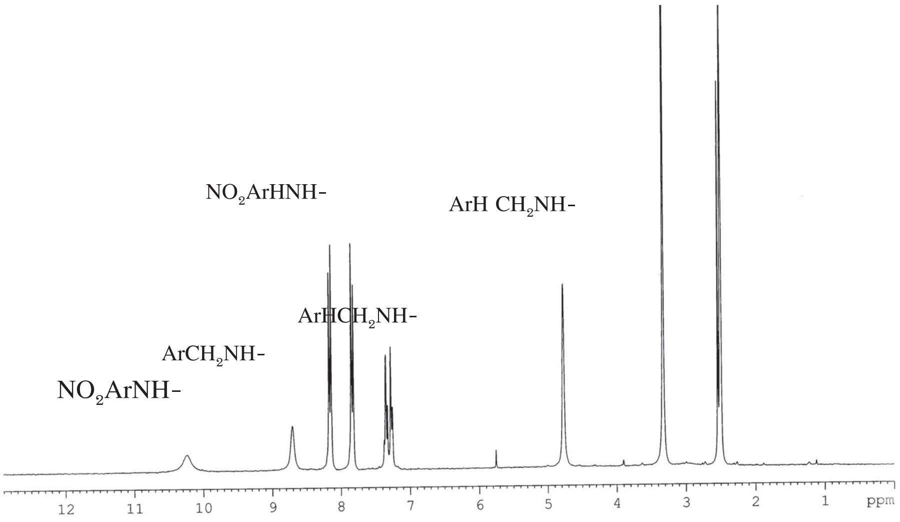

Figure 3 ^1H-NMR spectrum of **1**.

A standard ^{1}H-NMR titration experiment in DMSO-d_6 was performed using a constant host concentration and increasing concentration of various anions. The binding of anions was also clear from ^{1}H-NMR spectroscopy by monitoring the thiourea protons of **1** that initially appeared at 8.71 and 10.24 ppm (Figure 3). For F^- when 0.4 equiv. of tetrabutylammonium fluoride ($Bu_4N^+F^-$) was added the NH-protons thiourea moiety became broad and almost disappeared (Figure 4). The addition of excess F^- which caused disappearance of the NH-protons thiourea moiety was due to deprotonation and the formation of HF_2^- [12]. Thus, the binding constant of 1 towards F^- by ^{1}H-NMR titration could not be obtained.

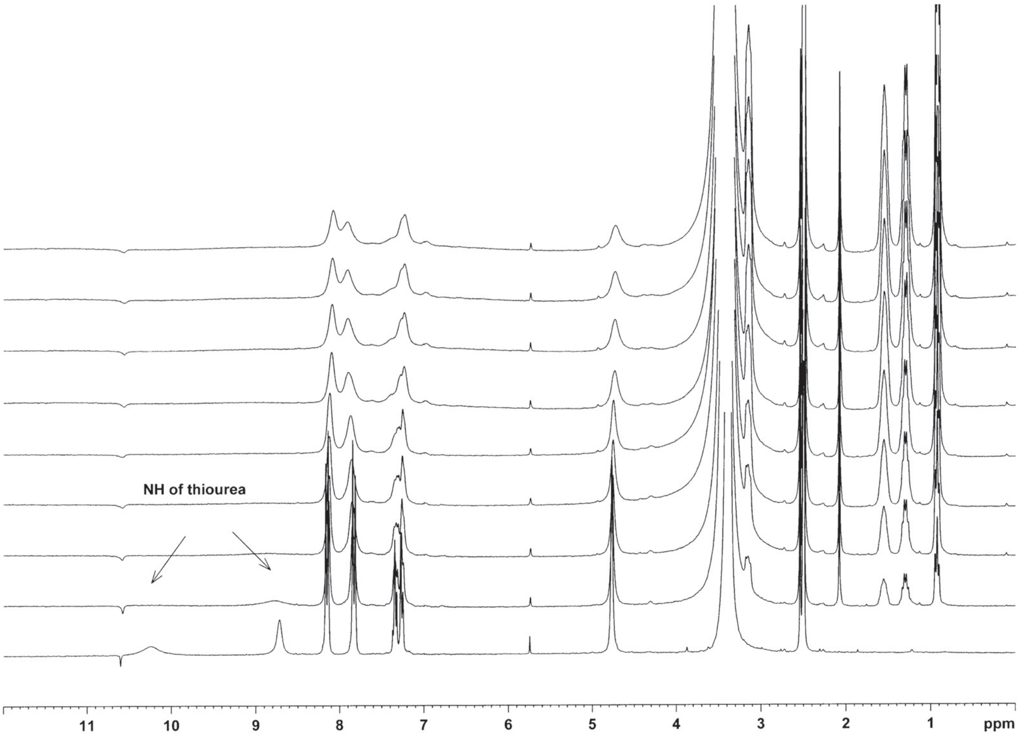

Figure 4 The downfield shift of NH resonances of thiourea moiety when F^- was added 0-2.0 equiv.

Addition of tetrabutylammonium salt such as $H_2PO_4^-$, HSO_4^-, Br^-, I^- and NO_3^- to a DMSO-d_6 solution of the compound **1** did not cause any shifts of the NH or other proton resonances which indicated that **7** could not form complex with HSO_4^-, Br^-, I^- and NO_3^-. However, the addition of tetrabutylammonium dihydrogenphosphate ($Bu_4N^+H_2PO_4^-$) and chloride ($Bu_4N^+Cl^-$) to a DMSO-d_6 solution of the compound **1** resulted in significant downfield shifts of the NH resonances ($\Delta\delta$ = 0.4-1.6) at room temperature (Figure 5 and 6), which was consistent with the formation of hydrogen-bonded complex. The NH-protons thiourea moiety at 10.24 ppm became broad and disappeared when 1.0 equiv. of $H_2PO_4^-$ was added, and then upon addition of a second equivalent, thiourea deprotonation was induced [13]. The plots between the mole fractions of $H_2PO_4^-$ (X) and the $\Delta\delta$ (1-X) are illustrated in Figure 7. Job's plot analysis indicated that **1** binds $H_2PO_4^-$ and Cl^- in a 1: 1 complex stoichiometry. The association constants of **1** towards $H_2PO_4^-$ and Cl^- calculated by the program EQNMR were shown in Table 4.

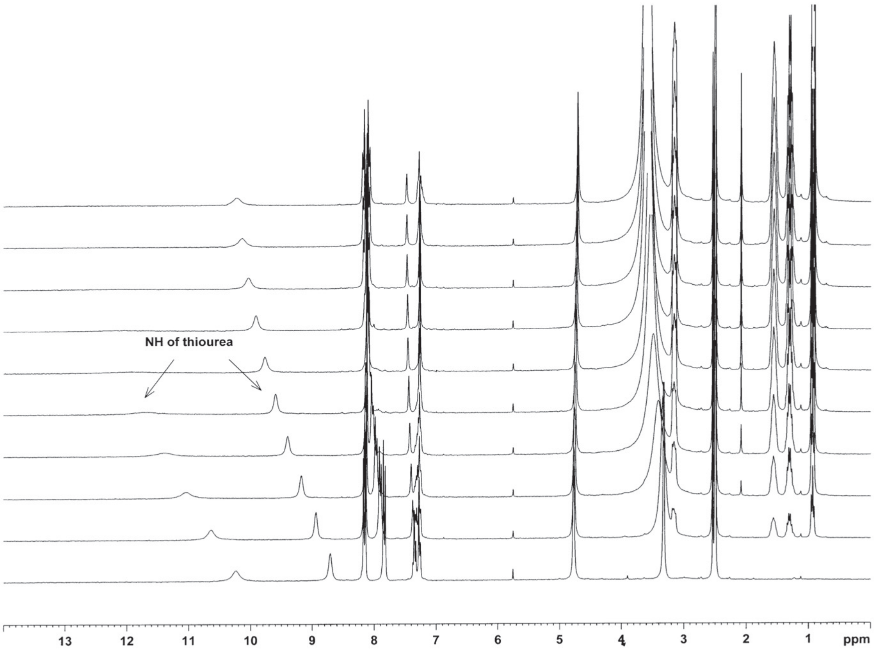

Figure 5 The downfield shift of NH resonances of thiourea moiety when $H_2PO_4^-$ was added 0-2.0 equiv.

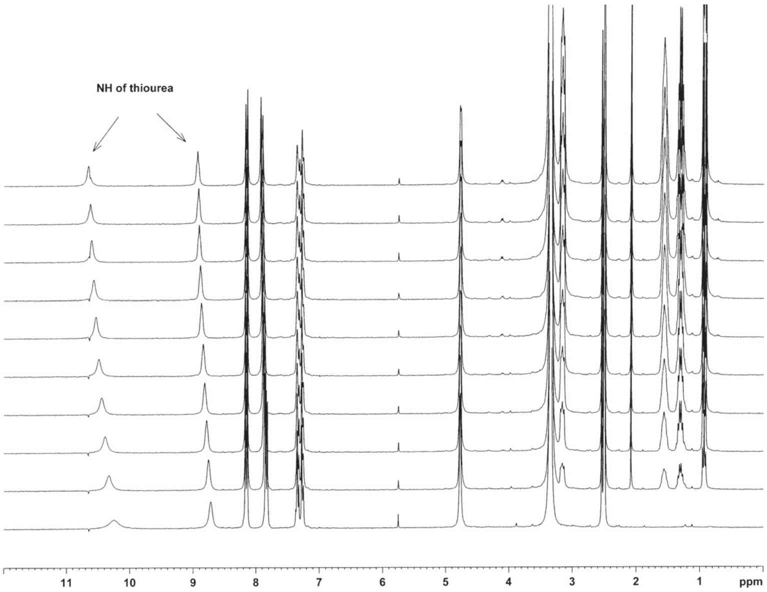

Figure 6 The downfield shift of NH resonances of thiourea moiety when Cl^- was added 0-2.0 equiv.

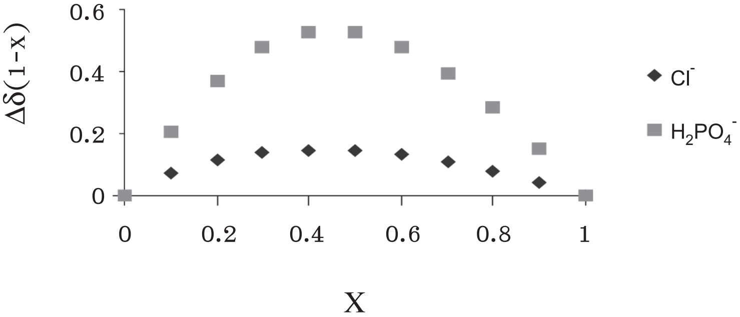

Figure 7 A 1: 1 complex stoichiometry of receptor **1** and $H_2PO_4^-$ or Cl^-.

Table 4 Association constants of compound **1** towards $H_2PO_4^-$, HSO_4^-, NO_3^-, Cl^-, Br^- and I^- using nBu_4N^+ as countercation.

Anion	pK_a^{a}	Association constants (M^{-1}) ^{b} **1**
$H_2PO_4^-$	2.16	136
Cl^-	-	44
Br^-	-	no binding
I^-	-	no binding
NO_3^-	-1.4	no binding
HSO_4^-	-3.1	no binding

Note: [a] In water at 25 °C

[b] All experiments were carried out at 298 K, errors estimated to be less than 15%

The results in Table 4 indicated that **1** could selectively bind with $H_2PO_4^-$ over Cl^-, but could not bind with Br^-, I^-, NO_3^- and HSO_4^-. The observed selectivity for $H_2PO_4^-$ could be rationalized based on the guest basicity and structure of $H_2PO_4^-$ containing four oxygens induced several. The oxyanion $H_2PO_4^-$ has four oxygens, suggesting that it may be bound with **1** and formed the strongest complex. The interaction between **1** and $H_2PO_4^-$ was shown in Figure 8. The oxyanions HSO_4^- and NO_3^- could bind to the host in similar manner as $H_2PO_4^-$. The structure of HSO_4^- is tetrahedron anion having four oxygens, while the structure of NO_3^- is trigonal planar anion having three oxygens. However, they are weak bases which cannot bind with host **1**. Br^- and I^- also possess the same basic property as NO_3^-. Therefore, the binding selectivity is explained in terms of the complex geometry and the basicity of the guest anions [14]. The tendency of binding selectivity between **1** and various anions exhibited in the order of $H_2PO_4^- >> Cl^- >>> Br^-$, I^-, NO_3^- and HSO_4^- in DMSO-d_6 by ^1H-NMR titration.

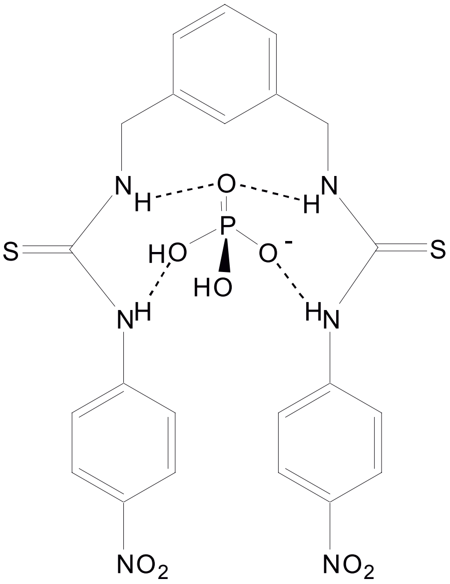

Figure 8 The strongest complex of 1 and $H_2PO_4^-$.

Chromogenic sensor 1 was also investigated with UV-Vis Spectroscopy. The effect of various anions in tetrabutylammonium salt (50 equiv.) on absorption spectrum of 1 was examined in DMSO and results were shown in Figure 9. Spectrum of 1 was measured in the absence of anions having UV-Vis spectrum with λ_{max} at 360 nm. UV-Vis spectrum of 1 with anions revealed different patterns. The anion sensor 1 did not show obvious spectra change even in the presence of NO_3^-, Br^-, I^- and HSO_4^- , while a slight response was observed after the addition of $H_2PO_4^-$, HSO_4^- and Cl^-, which supported the ability of complex formation 1 with the anions. So, the binding constant of 1 with anions by UV-visible titration will be investigated in the future. In contrast, significant change is observed in the presence of F^-, the intensity of the band at 360 nm decreased with little red shift of λ_{max} and a new band developed approximately at 485 nm turning color from yellow to orange (see color changes in Figure 10). A feature had been ascribed to the neat deprotonation of N-H fragments of the thiourea subunit [12, 13, 15, 16]. The selectivity trends of anion-induced color changes of 1 were determined as $F^- >>> H_2PO_4^-, HSO_4^-, NO_3^-, Cl^-, Br^-, I^-$.

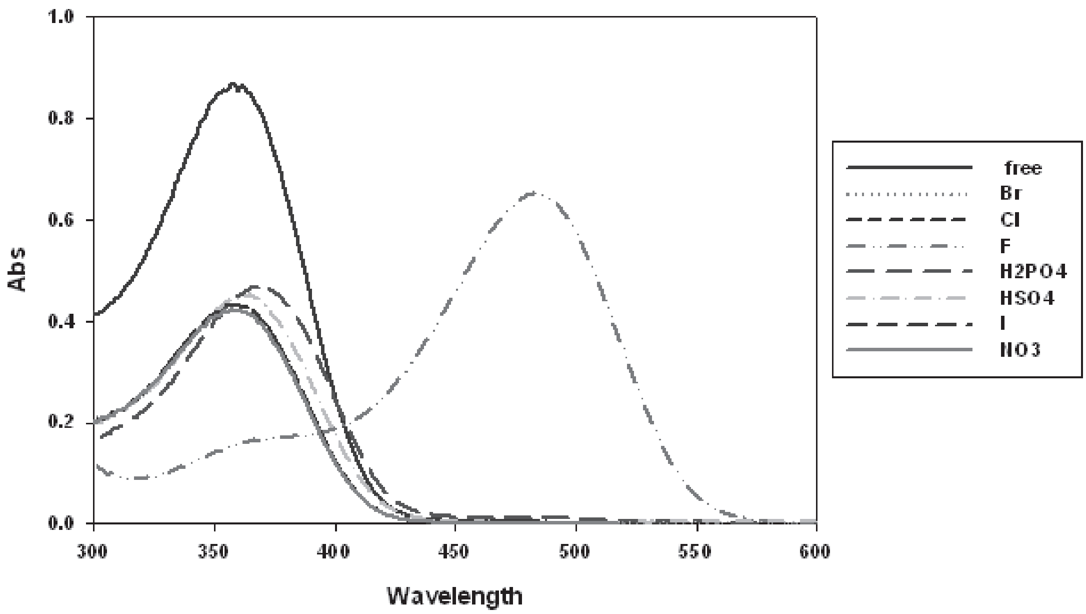

Figure 9 UV-Vis changes of 1 operated in DMSO (3 x 10^{-5} M) after the addition of 50 equiv. of anions.

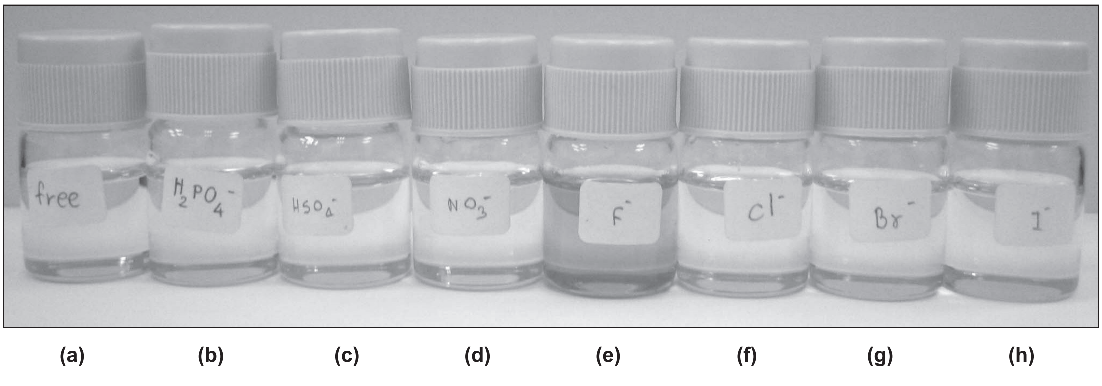

Figure 10 Color changes of 1 in DMSO [1] = (3 x 10^{-5} M) [anions] = 5 equiv.; (a) free; (b) H_2PO_4; (c) HSO_4^-; (d) NO_3^-; (e) F^-; (f) Cl^-; (g) Br^-; (h) I^-.

Conclusion

A new chromogenic anion sensor **1** was synthesized with 80% yield. Anions binding studies by ^{1}H-NMR titration method showed that **1** bound selectively with $H_2PO_4^-$ over Cl^-. In addition, selective and colorimetric sensing of F^- could be achieved by **1** in DMSO.

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ได้รับบทความวันที่ 1 สิงหาคม 2549
ยอมรับตีพิมพ์วันที่ 2 มีนาคม 2550