A unique NH-spacer for *N*-benzamidothiourea based anion sensors. Substituent effect on anion sensing of the ICT dual fluorescent *N*-(*p*-dimethylaminobenzamido)-*N*′-arylthioureas†

Fang-Ying Wu,[a,b] Zhao Li,[a] Lin Guo,[a] Xian Wang,[c] Meng-Hai Lin,[c] Yu-Fen Zhao[a] and Yun-Bao Jiang*[a]

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A series of *N*-(*p*-dimethylaminobenzamido)-*N*′-(substituted-phenyl)thioureas (substituent = *p*-CH_3, H, *p*-Cl, *p*-Br, *m*-Br, *m*-NO_2, and *p*-NO_2) were designed as anion sensors in order to better understand the –NH-spacer *via* a substituent effect investigation. In these molecules the dual fluorescent intramolecular charge transfer (ICT) fluorophore *p*-dimethylaminobenzamide as the signal reporter was linked to the anion-binding site, the thiourea moiety, *via* an N–N single bond. Correlation of the NMR signals of the aromatic and –NH protons with substituents in these molecules indicated that the N–N single bond stopped the ground-state electronic communication between the signal reporter and the anion-binding site. Dual fluorescence was observed in highly polar solvents such as acetonitrile with the former five derivatives. The fact that the CT emission wavelength and the CT to LE emission intensity ratio of the sensors were independent of the substituent existing in the anion-binding moiety suggested that the substituent electronic effect could not be communicated to the CT fluorophore in the excited-state either. Yet in acetonitrile both the CT dual fluorescence and the absorption of the sensors were found to be highly sensitive toward anions. A conformation change around the N–N bond in the sensor molecules was suggested to occur upon anion binding that established the electronic communication between the signal reporter and the anion-binding site. The anion binding constants of the *N*-(*p*-dimethylaminobenzamido)thiourea sensors were found higher than those of the corresponding traditional *N*-phenylthiourea counterparts and the substituent effect on the anion binding constant was much higher than that in the latter. "–NH–" was shown to be a unique spacer that affords *N*-benzamidothiourea allosteric anion sensors.

Introduction

A sensor molecule in general has a structural framework of "signal reporter–spacer–binding site", of which –CH_2– has been extensively employed as an efficient spacer especially in photo-induced electron transfer fluorescent sensors for metal cations and neutral species.[1] Recently this spacer has also been nicely extended to construct an anion spacer operating under the PET mechanism.[2] We have been interested in constructing thiourea-based ICT dual fluorescent sensors. It is known that the dual fluorescence due to the ICT of electron donor/acceptor substituted benzene derivatives has been shown to be sensitive to the strength of the electron donor and/or acceptor.[3] It is therefore possible to employ this character in constructing highly sensitive sensors[1,4–6] in particular those for anion sensing of current interest,[5] by a suitable combination of the CT fluorophore to the binding-site. With the ICT dual fluorescent sensors for anions, it is in principle optional that the anion-binding site is electronically coupled to the electron donor or acceptor of the dual fluorescent signal reporter in order to ensure an efficient and sensitive response based on the variations of the electron donating or accepting capability of the donor or acceptor resulting from anion binding. The way of linking the dual fluorescent signal reporter with the anion-binding site is therefore crucial, since this would also define the structural diversity of the dual fluorescent sensors that might eventually help to clarify structural elements necessary for an ICT dual fluorescent sensor. Our first attempt was to make *N*-(*p*-dimethylaminobenzoyl)thiourea (**1a**, Scheme 1) in which the thiourea anion-binding site is actually a part of the electron acceptor of the ICT dual fluorescent signal reporter, *p*-dimethylaminobenzamide (DMABA).[7] As thiourea is an important anion-binding receptor, it was natural to extend the investigation on **1a** to **1b** by introducing an *N*′-phenyl ring so that an additional substituent could be added to this phenyl ring to further tune the acidity of the thioureido –NH proton that is known to be important for anion binding.[5] Unfortunately, no response in both the absorption and fluorescence spectra of **1b** in acetonitrile was observed when anions such as acetate were introduced. An AM1 calculation (Scheme 2) and NMR data[8] suggested that this was due to an intramolecular hydrogen bond between the carbonyl oxygen and the thioureido –NH proton, known for *N*-acylthioureas.[9] The ^{1}H NMR data acquired in DMSO-d_6 indicated that the chemical shifts of the two thioureido –NH protons differed very much at 12.84 and 8.98 ppm, respectively.[8] The AM1 optimized structure indeed supported an intramolecular

[a]*Department of Chemistry and the MOE Key Laboratory of Analytical Sciences, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, 361005, China. E-mail: ybjiang@xmu.edu.cn; Fax: +86 592 218 5662; Tel: +86 592 218 5662*
[b]*Department of Chemistry, Nanchang University, Nanchang, 330047, China*
[c]*Department of Chemistry and the State Key Laboratory for Physical Chemistry of Solid Surfaces, Xiamen University, Xiamen, 361005, China*
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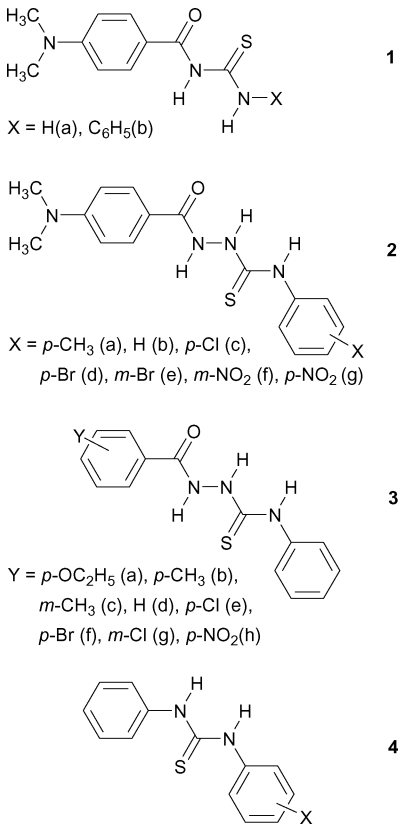
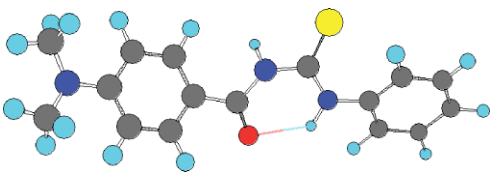
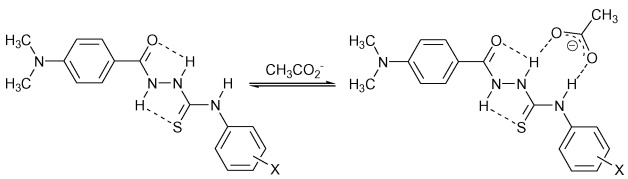


Scheme 1 Chemical structure of the thiourea-based anion sensors.

six-membered ring hydrogen bond with an O···H distance of 2.061 Å, Scheme 2. As a consequence of this intramolecular ring hydrogen bonding the expected double hydrogen bonding of the thiourea moiety with oxoanions (Scheme 3)[10] is not possible, resulting in no response in the spectra of **1b** toward anions.



Scheme 2 AM1 optimized structure of **1b**.



Scheme 3 Hydrogen bonding of **2** with acetate anion.[10]

It hence appeared reasonable to insert an atom in the amide C–N bond to prevent this favorable six-membered intramolecular ring hydrogen bond present in the acylthiourea sensor molecules. The first choice might be the well-employed $–CH_2–$ spacer.[1,2] Probably due to the saturated character of the sp^3 carbon in $–CH_2–$ that might stop the ground-state electronic communication between signal reporter and binding site, it has actually been shown that with the PET fluorescent sensors bearing a $–CH_2–$ spacer, the response toward sensing species is only shown in the fluorescence spectrum of the sensor whereas the absorption spectrum of the signal reporter remained unchanged. In view of taking the advantageous ICT dual fluorescent signal reporter, we turned to –NH–, in which the nitrogen atom bearing non-paired electrons might facilitate the electronic communication between the signal reporter and the binding site. *N*-(*p*-Dimethylaminobenzamido)-*N′*-phenylthiourea (**2b**, Scheme 1) was then designed,[10] in which the ICT fluorescent signal reporter, DMABA, was linked to the thiourea anion binding site *via* an "–NH–" spacer. It was found that this sensor showed a highly sensitive response toward anions such as AcO^- and F^- in *both* its dual fluorescence emission and absorption. Especially, the thiourea moiety in **2b** is linked to the DMABA signal reporter *via* an electron-donating nitrogen atom, the acidity of the thioureido –NH protons is therefore lower than that in **1a**, which should in principle lead to a lower anion binding affinity because of the hydrogen bonding interaction nature. The anion binding constant of **2b** in acetonitrile, at 10^6 mol^{-1} L orders of magnitude for AcO^- for example,[10] was actually much higher than that of **1a**.[7] This means that "–NH–" could be an interesting spacer in the thiourea-based anion sensor. Indeed, an extended investigation on *N*-(substituted-benzamido)thioureas (**3**, Scheme 1) with a variety of substituents other than *p*-$N(CH_3)_2$ indicated that the single N–N bond is twisted so that at least the ground-state electronic communication between the signal reporter and thiourea in **2b** and **3** is stopped.[11] This suggested that anion binding to the thiourea moiety in **2b** and **3** established, likely *via* a conformational change around the N–N single bond,[11] the ground-state electron communication between those two moieties in the sensor molecules. In order to better understand the unique –NH-spacer, we made a series of **2b** derivatives with a substituent at the *N′*-phenyl ring (**2a–g**, Scheme 1), as these molecules might emit the ICT characteristic dual fluorescence[3] so that the investigation could be carried out also by fluorescence monitoring in addition to absorption employed with **3**.[11] A substituent effect study into **2** did indicate that the –NH-spacer is unique in that *both* their fluorescence and absorption spectra undergo sensitive variations in the presence of an anion due to a conformational change occurring around the N–N bond and that both the anion affinity and the substituent effect on it with **2** are substantially higher than those with the corresponding traditional *N*,*N′*-diarylthioureas (**4**, Scheme 1). The *N*-benzamidothioureas (**2** and **3**) hence appear to be a set of allosteric sensors[5a,12] for anions.

Results and discussion

Twisted conformation of the N–N single bond in 2

The AM1 calculations and ^1H NMR data suggested the presence of the intramolecular hydrogen bonds in **2b** (Scheme 3, left),[10] yet our recent work on **3**[11] indicated that the N–N single bond in **2b** might also be twisted as in **3**, although the absorption spectral behavior of **2b** differed much from that of **3**. With a series of **2** available, it was found that the thiourea moiety in **2** was indeed electronically decoupled from the DMABA chromophore. Fig. 1 and 2 show the plots of the NMR signals of the aromatic and –NH protons in **2** as a function of the substituent at the *N′*-phenyl ring. It is seen in Fig. 1 that the NMR signals of the aromatic protons

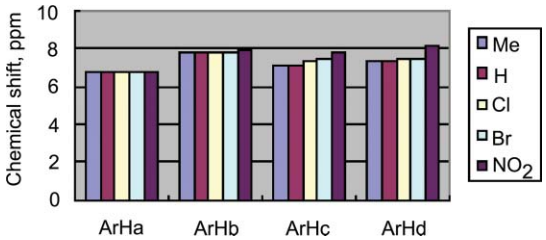


Fig. 1 ^{1}H NMR chemical shifts of the aromatic protons *versus* substituent.

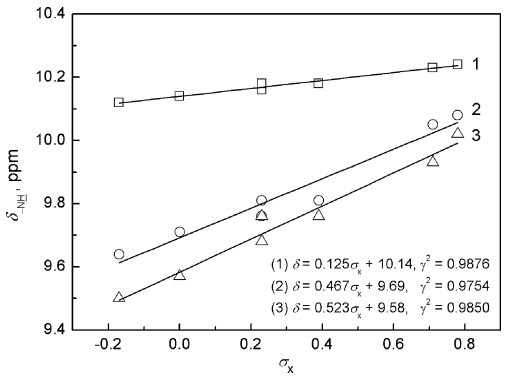


Fig. 2 Chemical shifts of the –NH protons in **2** *versus* the Hammett substituent constant. Line "1" corresponds to the NMR data of amido –NH protons and lines "2" and "3" correspond to those of the thioureido –NH protons, respectively.

at two phenyl rings show a dramatic difference in their response toward the substituent. While the NMR chemical shifts of the protons at the *N′*-phenyl ring bearing a substituent varied obviously with the substituent, those at the other phenyl ring remained silent. This means that the substituent effect could not be communicated to the other phenyl ring. Fig. 2 indicates that, while the NMR signals of the thioureido –NH protons show linear correlation with the Hammett constant[13] of the substituent by slopes of 0.523 and 0.467, respectively, those of the amido –NH protons show a much weaker dependence with a slope of only 0.125. Note that, in the case of **3** in which the substituent is located at the *N*-benzamido moiety, the NMR signal of the amido –NH proton showed a sensitive linear dependence on the Hammett constant with a slope of 0.463, whereas those of the thioureido –NH protons responded much less weakly toward the substituent with linear slopes of only 0.0977 and 0.128, respectively.[11] These observations suggest that it is the azino N–N single bond in **2** that is the stopper that makes the thiourea moiety, the anion-binding site, electronically decoupled from DMABA, the ICT dual fluorescent and absorption signal reporter.[11,14] This is in agreement with the highly twisted conformation of the N–N single bond reported for neutral hydrazines.[15]

The absorption spectra of **2a–g** and fluorescence spectra of **2a–e** in a variety of organic solvents were recorded and the main spectral data are given in Table 1. It was found that the absorption maxima of **2a–g** were almost independent of the substituent at the *N′*-phenyl ring and of the solvent polarity. Together with the observation of the molar absorption coefficients at 10^4 mol^{-1} L cm^{-1} orders of magnitude, it was assumed that the absorption of **2a–g** was due to a (π,π*) transition contributed

Table 1 Maximum absorption wavelength λ_{max}, molar absorption coefficient ε, the CT emission wavelength λ_{CT}, and fluorescence quantum yield Φ_F of **2a–g** in organic solvents

	Solvent[a]	λ_{max}/nm	ε, mol^{-1} L cm^{-1}	λ_{CT}, nm[c]	Φ_F
2a	DEE	310	5.0×10^4	—	0.239
	Dioxane	315	6.9×10^4	—	0.145
	THF	304	7.2×10^4	463	0.031
	EA	307	3.4×10^4	477	0.072
	$CHCl_3$	322	7.0×10^4	—	0.006
	CH_2Cl_2	320	6.6×10^4	—	0.025
	ACN	311	6.8×10^4	523	0.014
	MeOH	311	5.7×10^4	—	0.021
2b	DEE	301	2.3×10^4	—	0.076
	Dioxane	312	3.4×10^4	—	0.192
	THF	298	2.5×10^4	457	0.036
	EA	301	2.4×10^4	473	0.042
	$CHCl_3$	314	2.2×10^4	Shoulder	0.018
	CH_2Cl_2	310	2.2×10^4	460	0.034
	ACN	311	4.4×10^4	521	0.013
	MeOH	310	3.0×10^4	—	0.038
2c	DEE	306	3.4×10^4	—	0.110
	Dioxane	310	3.1×10^4	Tail	0.170
	THF	306	4.8×10^4	Tail	0.030
	EA	300	3.6×10^4	Tail	0.011
	$CHCl_3$	317	3.2×10^4	—	0.020
	CH_2Cl_2	318	3.7×10^4	—	0.103
	ACN	311	6.4×10^4	523	0.011
	MeOH	312	5.8×10^4	—	0.029
2d	DEE	306	1.9×10^4	—	0.213
	Dioxane	312	1.9×10^4	—	0.282
	THF	304	2.1×10^4	—	0.174
	EA	306	1.8×10^4	488	0.184
	$CHCl_3$	315	1.3×10^4	—	0.045
	CH_2Cl_2	317	1.5×10^4	—	0.062
	ACN	310	1.9×10^4	526	0.010
	MeOH	310	1.8×10^4	—	0.018
2e	DEE	308	1.4×10^4	—	0.180
	Dioxane	314	2.7×10^4	Shoulder	0.130
	THF	300	3.2×10^4	462	0.020
	EA	306	1.9×10^4	477	0.043
	$CHCl_3$	318	2.0×10^4	Shoulder	0.031
	CH_2Cl_2	317	1.2×10^4	458	0.176
	ACN	312	3.9×10^4	523	0.011
	MeOH	313	1.9×10^4	—	0.045
2f[b]	DEE	308	1.0×10^4		
	THF	309	1.7×10^4		
	EA	305	4.4×10^4		
	$CHCl_3$	324	2.8×10^4		
	ACN	313	4.5×10^4		
	MeOH	311	3.4×10^4		
2g[b]	DEE	311	2.8×10^4		
	EA	309	4.2×10^4		
	$CHCl_3$	324	4.3×10^4		
	ACN	315	4.6×10^4		
	MeOH	314	5.6×10^4		

[a] Solvent: DEE, diethyl ether; dioxane, 1,4-dioxane; THF, tetrahydrofuran; EA, ethyl acetate; $CHCl_3$, chloroform; CH_2Cl_2, dichloromethane; ACN, acetonitrile; MeOH, methanol. [b] **2f** and **2g** are nonfluorescent. [c] The position of the LE band at *ca.* 371 nm is not sensitive to either the substituent in **2a–e** or solvent.

mainly from the DMABA chromophore.[16] It hence follows that the chromophore does not have much electronic communication with the substituent at the *N′*-phenyl ring, in agreement with the conclusion reached from NMR data.

Dual fluorescence was observed with **2a–e** in a variety of polar solvents with the long-wavelength emission shifting to the red in highly polar solvents. This confirms the occurrence of the excited-state ICT with **2a–e** as assigned for DMABA[16] that the dual

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fluorescence is due to the locally excited (LE) state and the charge transfer (CT) state in equilibrium.[3] It was found that the position of the observed long-wavelength CT emission of **2a–e** in the same solvent showed no obvious dependence on the substituent at the *N′*-phenyl ring within the electron acceptor (Table 1). Also, the CT to LE intensity ratio of **2a–e** in the highly polar solvent ACN, 2.3 ± 0.3, was noted independent of the substituent. This is in clear contrast with what was observed in the CT fluorescence of the related molecules such as aryl *p*-dimethylaminobenzoates and benzanilides in which the substituent located in the aryl electron donor/acceptor.[17] In the latter cases, electronic communication exists between the substituent and the electron acceptor/donor and their CT emission shows a monotonous shift with the electron donating/accepting nature of the substituent.[17] These observations in both the absorption and fluorescence spectra of **2** indicate that the substituent effect is not communicated to the CT fluorophore in both the ground- and excited-state. The signal reporter in **2** is shown electronically decoupled from the thiourea moiety, the anion-binding site. It might accordingly be expected that no or a minor response toward anions in the fluorescence and absorption spectra of **2** could be observed, yet we did observe a sensitive response in the fluorescence and absorption spectra of **2b** toward anions in acetonitrile.[10]

Highly sensitive response in the ICT dual emission and absorption: conformational change in 2 upon anion binding

It was actually found that it was not only the absorption and fluorescence spectra of **2b**, but also the dual fluorescence of **2a–e** and the absorption of **2a–g** in ACN that were highly sensitive to anions. Fig. 3 shows the absorption and fluorescence spectra of **2e** in ACN, as an example, in the presence of AcO^- of increasing concentration. Similar variations were found in the presence of F^- and $H_2PO_4^-$ and with other derivatives of **2e**. Previously it was shown by a variety of means including ^{1}H NMR and ESI-MS that these anions bound *via* hydrogen bonds to the thiourea moiety in **2b** with a well defined 1 : 1 stoichiometry (Scheme 3).[10,18] The observation of the isosbestic and isoemissive points in the absorption and fluorescence spectral traces, respectively (Fig. 3) supported the well defined compositions of the binding complexes and the 1 : 1 stoichiometry was further confirmed by the Job plot obtained for the **2b**/AcO^- system in acetonitrile, Fig. 4.

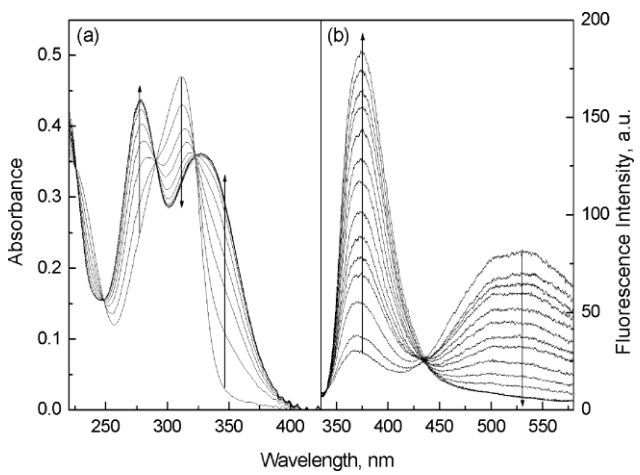

Fig. 3 Absorption (a) and fluorescence (b) spectra of **2e** in acetonitrile in the presence of an increasing amount of AcO^-. [**2e**] = 2.0×10^{-5} (a) and 1.0×10^{-5} (b) mol L^{-1}. Excitation wavelength employed to record spectra in (b) was 289 nm, an isosbestic wavelength seen in (a). Acetate and other anions examined existed in their tetrabutylammonium salts.

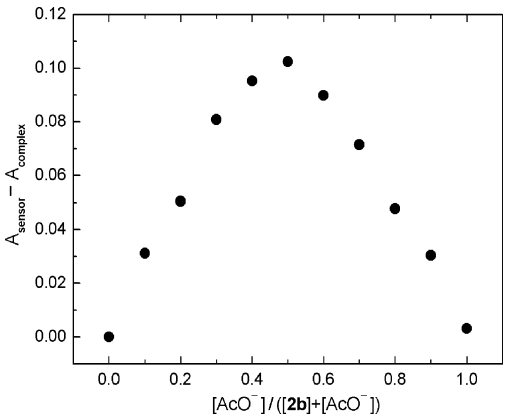

Fig. 4 Job plots for the binding of **2b** with AcO^- in acetonitrile. $A_{complex}$ and A_{sensor} are the absorbances at 311 nm of the AcO^-/**2b** mixture and **2b**, respectively. The total concentration of AcO^- and **2b** is 4.0×10^{-5} mol L^{-10}.

The observed sensitive response is significant since the anion binding site in **2** was shown to be electronically decoupled from the signal reporter (DMABA), which would have led to no or a minor spectral response toward anions. The dual emission character of the sensors helped in understanding the sensing mechanism. The variations in the dual fluorescence were characterized by the appearance of an isoemissive point during anion titration, while both the LE and CT band positions remained unchanged (Fig. 3b).[10] This indicated that anion binding to the thiourea moiety in **2a–e** stopped the CT process that is one of the depopulation channels of the LE state.[3] The CT to LE intensity ratio of **2a–e** was therefore decreased with increasing anion concentration. This might not be understood under the classic PET mechanism[1] since that would lead to the total quenching of the dual fluorescence.[10] Control experiments with thiourea-free model molecules of **2**, DMABA and *p*-dimethylaminobenzoylhydrazine, indicated that their CT dual fluorescence in ACN was not affected by either AcO^- or a diphenylthiourea–AcO^- complex,[10] confirming that no PET fluorescence quenching occurs by these anionic species. In PET fluorescent sensors where the thiourea moiety was linked to the anthracene chromophore *via* a "CH_2" spacer,[2d,e] it was shown that, while the anthracene fluorescence was quenched due to enhanced PET by anion binding, the absorption spectrum of anthracene remained unchanged. Therefore, the observed changes in *both* the absorption and dual fluorescence of **2** upon anion binding actually pointed to the establishment of the electronic communication between DMABA, the signal reporter, and the anion-binding thiourea moiety. This means that a conformational change around the N–N bond in **2** occurs when binding to the anion.

It was noted that in the presence of an anion, the absorption maxima of **2a–f** in ACN were shifted to 328 nm.[19] This band position is in between those of DMABA and **1** (Scheme 1) of 300 nm and 345 nm, respectively. Note that before anion binding the absorption maxima of **2** were at *ca.* 310 nm (Table 1) that is close to that of DMABA.[16] Obviously the absorption of **2** after anion binding becomes closer to that of **1** in which the thiourea

moiety is electronically conjugated to the DMABA chromophore. This provides additional support to the assumption of the anion-binding inducing conformational change around the N–N bond in **2** that makes the thiourea moiety in **2** somewhat coupled electronically to the DMABA chromophore.[11,20] As a consequence, the electron acceptor in **2** would be substantially weakened when the anion binds to the thiourea moiety in it, which disfavors the excited-state CT process, leading to attenuated CT emission while concomitantly enhanced LE emission.

Anion binding affinity of 2

Anion binding constants of **2a–g** in ACN were evaluated by nonlinear fittings of the variations of both the absorption and dual fluorescence intensity ratio (I_{CT}/I_{LE}) of **2** *versus* anion concentration.[10,21] From the data presented in Table 2, it was found that in general the anion-binding constant of **2** increases when the *N′*-phenyl substituent is more electron-withdrawing. This means that the acidity of the thioureido –NH proton indeed governs the anion binding of the thiourea-based sensors.[5] The observation that the binding constant for AcO^- was higher than those for F^- and $H_2PO_4^-$ (Table 2), in the order of anion basicity, agreed with this conclusion. Because of the similar geometry of the thiourea moiety in **2** and of the AcO^- anion with the same sp^2 carbon center, binding constants for AcO^- of **2** were correlated to the Hammett constant of the substituent.[13] Linear correlation[22] was found with slopes of 8.74 and 10.57 from the binding data obtained in fluorescence and absorption titrations, respectively (Fig. 5). It is significant to note that the anion-binding constants of **2**, with AcO^- for example, are higher than not only that of **1a** but in particular higher than those of the corresponding *N′*-(substituted-phenyl)thiourea counterparts (**4**, Scheme 1) that are at 10^3–10^4 mol^{-1} L orders of magnitude (Fig. 5), despite the lower acidity of the thioureido –NH protons in **2** compared to that in **4**. The latter is suggested by the chemical shifts of the thioureido –NH protons in **2** in comparison with those of **4** shown later in the text. A linear correlation was also found between the binding constants of **4** with AcO^- and the Hammett substituent constant, but with a much lower slope of only 2.52, Fig. 5. It is therefore made clear that anion binding to **2** not only establishes the electronic communication between the DMABA signal reporter and the anion-binding site in **2**, but, more importantly, the anion-binding induced conformational change in **2** substantially enhances the *N′*-phenyl substituent effect on the anion binding. This observation is significant since, in these two kinds of sensors (**2** and **4**, Scheme 1), the substituent is connected in the same manner to the thiourea moiety. The NMR chemical shifts of the thioureido –NH protons in the available derivatives of **4** were measured in DMSO-d_6 and correlated with the Hammett substituent constant in the same manner as with **2** as shown in Fig. 2. The linear relationships found for **4** were $\delta_{\text{-NH(a)}} = 0.693\ \sigma_X + 9.75$ ($n = 6$, $\gamma^2 = 0.9689$) and $\delta_{\text{-NH(b)}} = 0.546\ \sigma_X + 9.77$ ($n = 6$, $\gamma^2 = 0.9806$), respectively. Here –NH(a) represents the –NH proton of the aniline bearing the substituent and –NH(b) the other thioureido –NH proton. Compared to those of the corresponding relationships of **2** given in Fig. 2, it is obvious that the linear Hammett-constant dependence of the ^{1}H NMR signals of the thioureido –NH protons in **2** and **4** is similar. Therefore, the difference in the substituent effect on the anion binding affinity of **2** and **4** shown in Fig. 5 is not due to the difference in the substituent effect on the acidity of the thioureido –NH protons that has been shown to be important in governing the anion binding of thiourea-based sensors. A comparison of their NMR chemical shifts indicated that the acidity of the thioureido –NH protons in **2** is actually lower than that in **4**, which should have led to lower anion binding affinities of **2** because of the hydrogen bonding nature of the sensor–anion interaction.[5] In fact, it was observed that the anion binding affinities of **2** were higher than

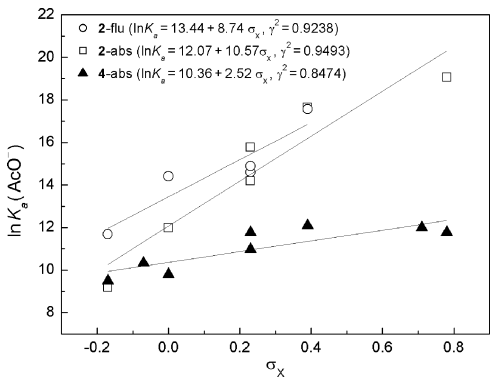

Fig. 5 Semilogarithm plots of the AcO^- binding constants of **2** and **4** against the Hammett constant of the substituent in **2** and **4**. "Flu" means that the binding constants were obtained from the dual fluorescence intensity ratio and "abs" means the binding constants fitted from variations in the absorption of the sensor.

Table 2 Binding constants in ACN obtained from fluorescence and absorption titrations[a]

	K_a/mol^{-1} L					
	AcO^-		F^-		$H_2PO_4^-$	
	Flu[b]	Abs[c]	Flu[b]	Abs[c]	Flu[b]	Abs[c]
2a	7.1×10^4	9.7×10^3	2.9×10^4	9.0×10^3	1.9×10^4	2.1×10^4
2b	1.8×10^6	1.6×10^5	2.1×10^5	4.9×10^4	1.5×10^5	1.3×10^4
2c	2.2×10^6	1.5×10^6	3.3×10^5	3.6×10^4	4.7×10^5	—
2d	2.9×10^6	2.5×10^6	5.9×10^5	2.8×10^4	8.6×10^5	1.5×10^5
2e	4.3×10^7	4.6×10^7	4.4×10^5	6.0×10^4	4.7×10^5	3.8×10^4
2f	—	2.9×10^6	—	1.2×10^5	—	1.1×10^5
2g	—	1.9×10^8	—	7.8×10^4	—	1.3×10^5

[a] Measurements for the binding constants for Cl^-, ClO_4^-, Br^-, and NO_3^- were also tried but the constants were too low to be determined. [b] Binding constants obtained by fitting the CT to LE dual fluorescence intensity ratio. [c] Binding constants fitted from absorption titrations.

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those of **4** (Fig. 5). This means that the anion-binding induced conformational change around the N–N single bond in **2** has a positive feedback to the anion binding that also amplifies the effect of the *N′*-phenyl substituent on the anion affinity. A cooperative effect in anion binding was therefore inferred to accompany the anion-binding induced conformation change in **2**. This suggests that **2** is a kind of allosteric sensor[5a,12] for anions. Referring to the intra- and intermolecular hydrogen bond network in the anion–**2** binding complex (Scheme 3, right), it is obvious that, upon anion binding to the thiourea moiety in **2**, the electron density at the thiourea S atom increases, which would enhance its hydrogen bonding with the amido –NH proton. Note that the thiourea moiety in this binding complex acts as both the hydrogen bonding proton donor and acceptor, a cooperative effect would exist that enhances the hydrogen bonding as is seen in the hydrogen bonding of nucleic bases (A–T and G–C) in their pairing to define the double helix structure of DNA and in related artificial hydrogen bonding systems.[23] Subject to the directionality of the hydrogen bond,[24] the anion–**2** binding complex around the amidothiourea unit would take a relatively planar conformation to accommodate the hydrogen bonding network. This might explain why the thiourea moiety in **2** becomes somewhat conjugated with the DMABA fluorophore when the anion is bound and the substantially enhanced substituent effect on the anion binding constants of **2**. Extended experiments are being undertaken to further clarify the detailed mechanism.

Conclusions

The N–N single bond in a series of *N*-(*p*-dimethylaminobenzamido)-*N′*-arylthioureas (**2a–g**) was shown to be highly twisted that makes the DMABA fluorophore electronically decoupled from the thiourea anion-binding site in both the ground and excited states. Yet, a highly sensitive response in *both* the ICT dual fluorescence of **2a–e** and absorption of **2a–g** was observed toward anions such as AcO^-, $H_2PO_4^-$, and F^- in ACN. It was concluded that a conformational change occurred in **2** around the N–N single bond when they bind to the anion that made the signal reporter and the anion-binding site electronically coupled. A direct consequence of this conformational change was the substantially enhanced anion binding affinity of the neutral thiourea based sensors and, in particular, the substantially enhanced *N′*-phenyl substituent effect that tuned the anion binding affinity of the sensors. The fact that the anion binding affinity of *N*-(substituted-benzamido)-*N′*-phenylthioureas (**3**, Scheme 1), in which the substituent locates at the *N*-benzamido phenyl ring is independent of the substituent,[11] suggests that it is the substituent at the *N′*-phenyl ring in **2** that initializes the substituent effect in tuning the anion affinity of the *N*-(substituted-benzamido)-*N′*-(substituted-phenyl)thiourea based anion sensors. The "–NH–" spacer therefore appears to be unique for constructing thiourea-based anion sensors of high anion binding affinity not achieved by the traditional means of increasing the acidity of the thioureido –NH protons. It is therefore expected that, by taking DMABA, for example, as the CT dual fluorescent signal reporter, together with a sophisticated choice of substituent(s) and/or additional binding site(s) at the *N′*-phenyl ring, smart *N*-(*p*-dimethylaminobenzamido)-*N′*-arylthiourea based ratiometric allosteric sensors for anions might be achieved, selective for given anions and operative in highly competitive solvents such as water.

Experimental

Absorption spectra were taken on a Varian Cary 300 spectrophotometer and corrected fluorescence spectra were recorded on a Hitachi F-4500 spectrophotometer. Fluorescence quantum yields were measured using quinine sulfate as a standard ($\Phi_F = 0.546$ in 0.05 N H_2SO_4).[25] ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were acquired in DMSO-d_6 using TMS as the internal standard. HRMS were obtained by injection of a methanol solution of the sample. Fluorescence and absorption spectral titrations for anion binding were carried out by adding an aliquot of the anion solution into a bulk sensor solution at a given concentration.

Solvents used for the synthesis of sensors were available on the market at AR (analytical reagent) grade. Solvents for spectral investigations were purified by re-distillation until no fluorescent impurity could be detected. Tetrabutylammonium salts of the anions were prepared by neutralization of the corresponding acids with tetrabutylammonium hydroxide. *N*-(*p*-Dimethylaminobenzamido)-*N′*-arylthioureas (**2**, Scheme 1) were synthesized from the reactions in ethanol at room temperature of *p*-(dimethylamino)benzoylhydrazine with (substituted-phenyl)isothiocyanates and were purified by repeated recrystallizations from absolute ethanol. Full characterization data are supplied in the supplementary data.† Derivatives of **4** were those available in our laboratory.

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