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Detailing Hydrogen Bonding and Deprotonation Equilibria between Anions and Urea/Thiourea Derivatives

Carol Pérez-Casas and Anatoly K. Yatsimirsky*

Facultad de Química, Universidad Nacional Autónoma de México, 04510 México D.F., Mexico

anatoli@servidor.unam.mx

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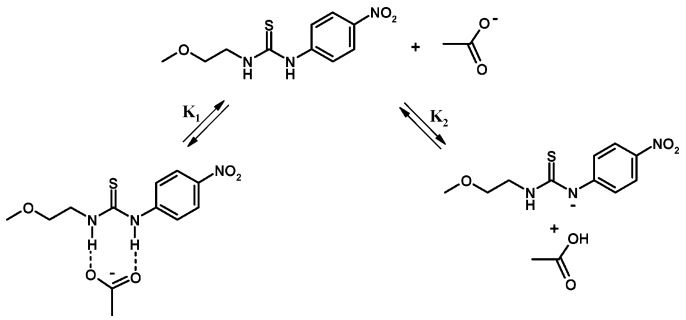

Spectrophotometric and ^{1}H NMR titrations of *N*-methoxyethyl-*N*′-(4-nitrophenyl)thiourea (**3**) by Bu_4-NOAc show that in DMSO deprotonation of the receptor and formation of a hydrogen-bonded complex with anion proceed simultaneously but in MeCN deprotonation requires the participation of the second acetate anion. The formation constants of hydrogen-bonded complexes were determined from titrations in the presence of added acetic acid, which suppressed deprotonation. These constants together with independently measured stability constants of $(AcO)_2H^-$ complexes were employed for a rigorous numerical analysis of titration results in the absence of added acid, which allowed us to determine the equilibrium deprotonation constants as well as pK_a values for **3** in both solvents. Although **3** appeared to be a weaker acid than AcOH in both solvents, it can be deprotonated by acetate in dilute solutions when the concentration of liberated acetic acid is low enough. With disubstituted *N*,*N*-bis(methoxyethyl)-*N*′-(4-nitrophenyl)thiourea **4** only deprotonation equilibrium is observed. In contrast, both parent urea derivatives **1** and **2** cannot be deprotonated by acetate anions. Independent of the real type of equilibrium, whether it is a deprotonation or a hydrogen bonding, titration plots always can be satisfactorily fitted to a formal 1:1 binding isotherm. A relationship between apparent "binding constants" and real equilibrium constants of hydrogen bonding association and deprotonation processes is discussed.

Introduction

Anion recognition by hydrogen-bonding receptors is an area of intensive current research.[1] A particularly important characteristic of anion receptors required for their applications as sensors is their ability to signal the interaction with anions in an easily detectable and quantifiable way. For this reason, the discovery of capacity of some anions to abstract protons from sufficiently acidic receptors converting them into brightly colored deprotonated forms attracted much attention.[2−5] Although in this process the receptor behaves essentially as an acid−base indicator, there is a significant selectivity to the type

* To whom correspondence should be addressed. Tel: 55 5622 3813. Fax: 55 5616 2010.

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of anion, and several systems based on this phenomenon have been developed for practical anion sensing.[6]

A recently developed general scheme of processes involved in anion−receptor interaction considers three types of reactions.[2] If basicity of an anion A^- is insufficient to induce deprotonation of the receptor RH, one observes formation of a hydrogen-bonded complex $R-H\cdots A^-$ (eq 1) manifested in a red shift of the receptor UV absorption band and a downfield shift or often disappearance of NMR signals of receptor protons involved in the hydrogen bonding.

$$RH + A^- \rightleftarrows RHA^- \quad (1)$$

If basicity of an anion if high enough to deprotonate the receptor (eq 2), one observes appearance of a new intense absorption bond in the visible range of the electronic spectrum attributed to the deprotonated receptor, disappearance of NMR signals of abstracted receptor protons, and an upfield shift of signals of adjacent receptor protons.

$$RH + A^- \rightleftarrows R^- + AH \quad (2)$$

In a borderline case, an anion initially forms a hydrogen-bonded complex, but with sufficiently high excess of added anions the deprotonation occurs due to formation of hydrogen bonded anion dimers A_2H^- (eq 3), which shifts the equilibrium 2 to the right. This last situation is observed most often with fluoride anion and also was described with acetate anions.[2a,b,e]

$$AH + A^- \rightleftarrows A_2H^- \quad (3)$$

The respective equilibrium constants are given by eqs 4−6.

$$K_1 = [RHA^-]/[RH][A^-] \quad (4)$$

$$K_2 = [R^-][AH]/[RH][A^-] \quad (5)$$

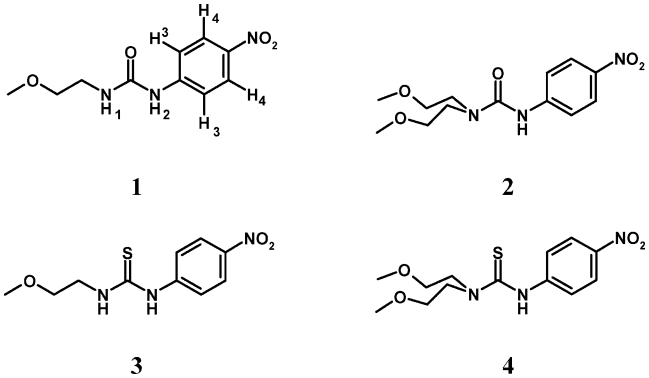
$$K_3 = [A_2H^-]/[AH][A^-] \quad (6)$$

Although this scheme in general is well justified, a quantitative description of equilibria involved still requires more detailing. In particular, the deprotonation process is always characterized in terms of a meaningless "binding" constant formally calculated by fitting the absorbance changes as a function of added anion concentration to the equation for 1:1 binding isotherm and the real K_2 value has never been estimated. Also, in situations when a second anion is needed to induce the deprotonation, the fact that the complex A_2H^- has a sufficiently high formation constant has never been proved, although the reaction is usually performed in highly polar solvents like DMSO where such complexes may be rather unstable. Finally, a situation when both formation of a hydrogen-bonded complex and deprotonation processes occur simultaneously was not yet analyzed quantitatively. This last situation is important because the highest stability of hydrogen-bonded complexes is reached for couples of proton donor and proton acceptor of equal basicity,[7] but for such a couple $K_2 = 1$ and deprotonation by 50% should be observed already in equimolar mixture of anion and receptor. However, in fact, the degree of deprotonation is a more complex function of equilibrium constants and reaction conditions. It follows from eqs 4 and 5 that the ratio of concentrations of deprotonated and hydrogen bonded forms is given by eq 7.

$$[R^-]/[RHA^-] = K_2/K_1[AH] \quad (7)$$

Obviously, strong hydrogen bonding (large K_1) will reduce the degree of deprotonation. At the same time, if there is no added acid the concentration of AH will be equal to the concentration of deprotonated form, which in its turn is proportional to total receptor concentration. Therefore, deprotonation will be more significant in more dilute receptor solutions where less acid is produced and the "binding constant" formally calculated for the deprotonation reaction will depend on the receptor concentration.

In this paper, possible approaches to analyze all of these aspects are illustrated by performing a detailed study of interactions between acetate anions and a set of receptors **1**−**4** of structural type frequently employed in design of anion-sensing molecules in two most often used solvents DMSO and MeCN. Both UV−vis and NMR titrations were analyzed numerically in terms of a complete set of coexisting equilibria 1−3. An approach to determine separately the equilibrium constant for the hydrogen-bonding reaction by performing titrations in the presence of added acid was applied. Special attention was paid to consistency of spectroscopic manifestations of one or another type of interaction, which follow from the results of UV−vis and NMR titrations.



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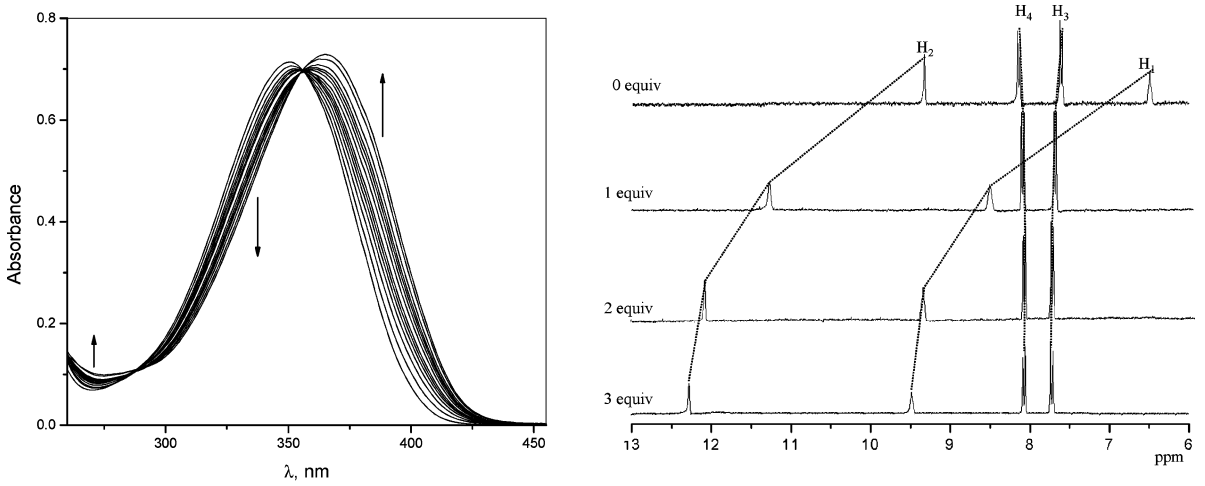

FIGURE 1. (A) Spectrophotometric titration of 4.8×10^{-5} M **1** by Bu_4NOAc in DMSO. Arrows show the directions of spectral changes at increasing Bu_4NOAc concentrations (0−4 mM). (B) Partial ^1H NMR spectra of 5 mM **1** in DMSO-d_6 recorded with increased amounts of Bu_4NOAc.

TABLE 1. Summary of Equilibrium Constants for Hydrogen Bonding or/and Deprotonation of Receptors 1−4 and Acetic Acid by Acetate Anions

	K_1, M^{-1}		K_2		K_3, M^{-1}	
receptor	DMSO	MeCN	DMSO	MeCN	DMSO	MeCN
1	$(1.09 \pm 0.05) \times 10^3$	$(2.15 \pm 0.07) \times 10^4$				
2	40 ± 10	14 ± 2				
3	$(1.14 \pm 0.03) \times 10^3$	$(1.06 \pm 0.06) \times 10^5$	0.050 ± 0.05	0.020 ± 0.002		
4			2.0 ± 0.2	0.040 ± 0.004		
AcOH					125 ± 4	$(9.7 \pm 0.2) \times 10^3$

Results and Discussion

Urea Derivatives 1 and 2. Arylurea derivatives lacking strong electron-accepting substituents usually cannot be deprotonated by anions.[2a] In line with this, we observed with compounds **1** and **2** only formation of hydrogen-bonded complexes with acetate. Figure 1A illustrates the course of spectrophotometric titration of **1** by Bu_4NOAc in DMSO, which shows a typical pattern for formation of a hydrogen-bonded complex. The absorption maximum of **1** at 351 nm shifts to 365 nm with two isosbestic points at 356 and 288 nm. The spectral course of interaction of **1** with acetate anions in MeCN was similar but all spectra were shifted to shorter wavelengths by ca. 20 nm.

Fitting the profiles of absorbances (Abs) measured at several fixed wavelengths vs [Bu_4NOAc] to the eq 8 for a 1:1 binding isotherm (subscript T stands for total concentration, Abs_0 is the initial absorbance measured in the absence of acetate, $\Delta\epsilon$ is the difference in molar absorptivities between the complex and free receptor)[8] gives $K_{assoc} = K_1$ shown in Table 1. Examples of such fittings are shown in Figure 1S(A) (Supporting Information). As expected for a hydrogen-bonded association, larger K_1 is observed in a less polar solvent.

$$\mathrm{Abs} = \mathrm{Abs}_0 + 0.5\Delta\epsilon([\mathrm{RH}]_T + [\mathrm{AcO}^-]_T + 1/K_{assoc} - (([\mathrm{RH}]_T + [\mathrm{AcO}^-]_T + 1/K_{assoc})^2 - 4[\mathrm{RH}]_T[\mathrm{AcO}^-]_T)^{0.5}) \quad (8)$$

Results of NMR titration confirm this type of interaction. Figure 1B shows the partial ^1H NMR spectra of **1** recorded with increased amounts of acetate in DMSO-d_6, from which one can see that signals of ureido protons H_1 and H_2 undergo a strong downfield shift by ca. 3 ppm, signals of H_3 also undergo a small downfield shift by 0.1 ppm attributable to a through-space interaction with hydrogen bound acetate anion,[2c] and signals of H_4 undergo also small, but upfield shift due to an inductive shielding effect of the bound anion. Fitting of the titration profile for H_2 protons, Figure 1S(B) (Supporting Information), to the equation similar to 8, but rewritten for NMR titration, gives $K_1 = 720 \pm 50$ M^{-1} reasonably close to the value found by more precise spectrophotometric titration. Similar pattern was observed also in MeCN, but in this case, K_1 was too large to be reliably calculated form NMR results obtained at high receptor concentration.

Interaction of receptor **2** with acetate in both solvents also proceeds as hydrogen-bonding association (Figure 2S, Supporting Information), but with expectedly much smaller K_1 values found by NMR titrations (Table 1).

Association between Acetic Acid and Acetate Anion. The association constants $K_3 = 180$ M^{-1} in DMSO and 1600−4000 M^{-1} in MeCN have been determined recently by potentiometric titrations with a glass electrode and a modified calomel electrode.[9] Since the use of a glass electrode in pure nonaqueous solvents is not free of possible systematic errors and the calculations were based on literature values of pK_a of picric acid in respective solvents used as a standard for the electrode

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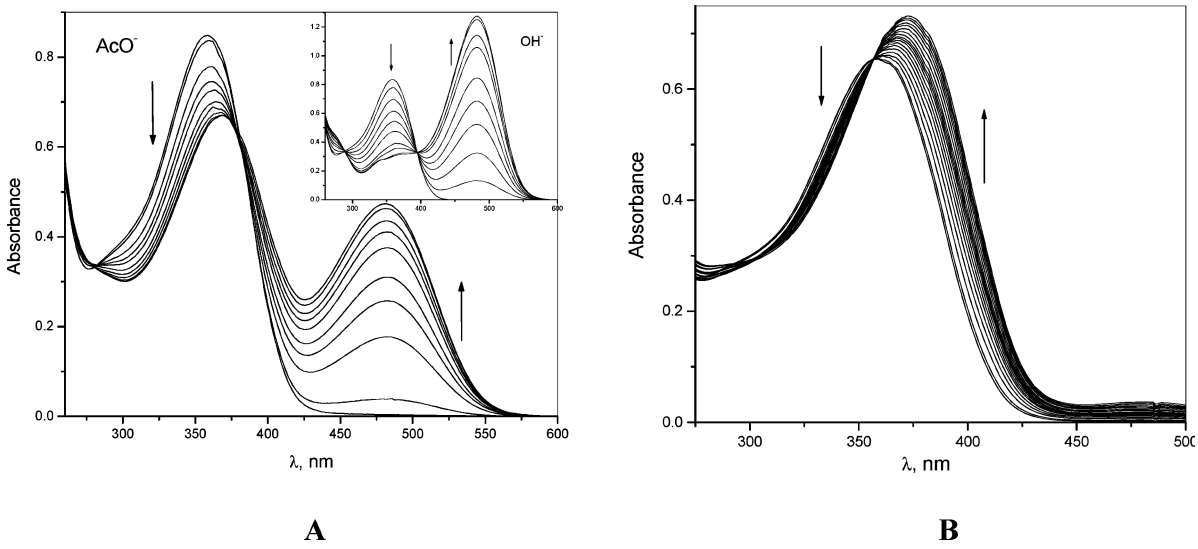

FIGURE 2. (A) Spectrophotometric titration of 7.0×10^{-5} M **3** by Bu_4NOAc (0−0.8 mM) and Bu_4NOH (0−0.4 mM) in DMSO. (B) Spectrophotometric titration of 5.0×10^{-5} M **3** by Bu_4NOAc (0−3 mM) in DMSO in the presence of 1.9 mM AcOH. Arrows show the directions of spectral changes at increasing Bu_4NX concentrations.

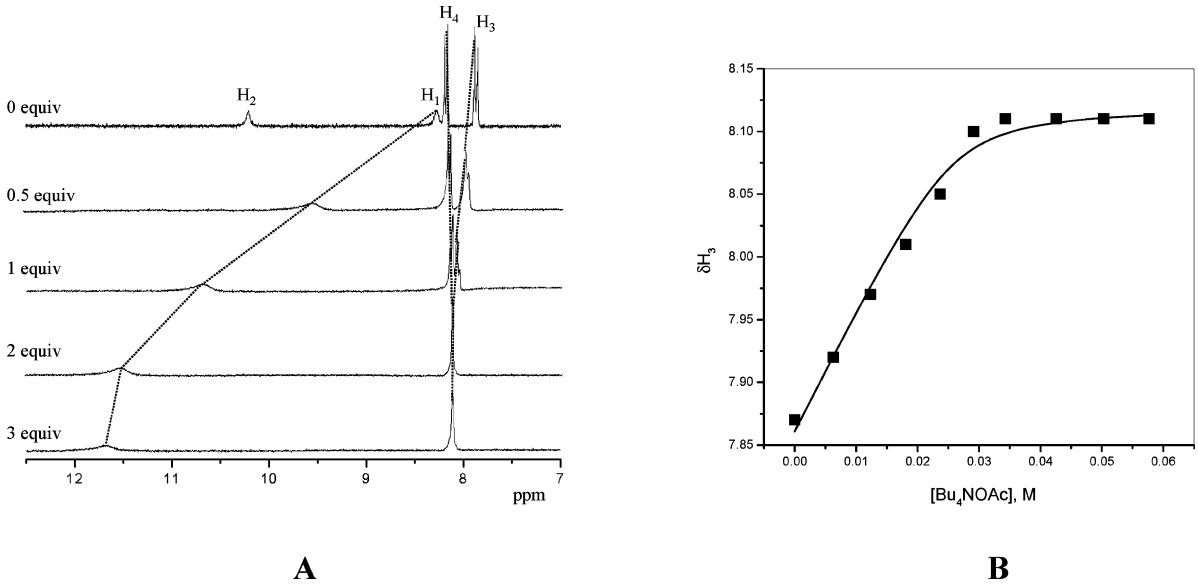

FIGURE 3. (A) Partial ^1H NMR of 24 mM **3** with increased amounts of added Bu_4NOAc in DMSO-d_6. (B) Titration profile for H_3, solid line is the fitting curve to the equation for 1:1 complexation.

calibration, we considered it worthy to determine K_3 values in these solvents again by an independent technique. An attempt to measure K_3 by ^1H NMR titration of acetic acid with Bu_4NOAc was unsuccessful because of strong broadening and disappearance of the signal of the carboxylic group proton already on addition of ca. 5% molar equiv of acetate ions. Therefore, we employed the effect of acetic acid on the interaction of acetate anions with **1**, which was not complicated by the deprotonation process, for an indirect determination of K_3.

In preliminary experiments, we found that addition of up to 0.1 M AcOH did not affect UV and NMR spectra of **1** in DMSO and MeCN. At the same time, addition of AcOH to a solution containing a mixture of **1** with an excess of acetate anion resulted in a restoration of the original spectrum of **1**. This can be attributed to displacement of equilibrium 1 to the left due to consumption of free acetate anions by acetic acid affording the complex $(AcO)_2H^-$ via reaction 3. Under conditions of a significant excess of Bu_4NOAc over **1** and of AcOH over Bu_4NOAc, a simple eq 9 can be used to fit the profile of absorbance at a fixed wavelength vs [AcOH] obtained in the presence of a constant concentration of Bu_4NOAc. In eq 9, A_{01} and A_{00} are the initial absorbance of the mixture of **1** with Bu_4NOAc and the absorbance of **1** alone respectively, and $K_{obs} = K_3/(1 + K_1[Bu_4NOAc])$.

$$A = (A_{01} + A_{00}K_{obs}[AcOH])/(1 + K_{obs}[AcOH]) \quad (9)$$

Figure 3S (Supporting Information) illustrates the titration profiles obtained in DMSO (3 mM Bu_4NOAc added) and MeCN (0.14 mM Bu_4NOAc added), which fit to eq 9 with observed equilibrium constants 29.1 ± 0.1 and 2720 ± 50 M^{-1}, respectively. With the above K_1 values, one obtains from these results the equilibrium constants K_3 for the reaction 3 given in Table 1, which are reasonably close to the above-reported

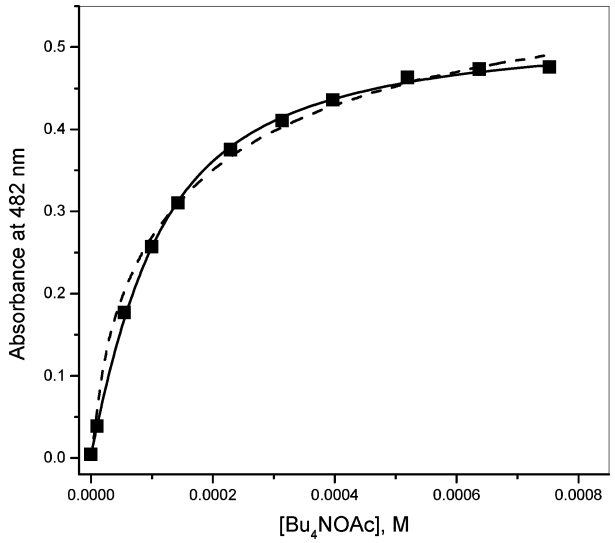

FIGURE 4. Titration curve for 7.0×10^{-5} M **3** (absorbance at 482 nm) with Bu_4NOAc in DMSO. Solid line: formal fit to eq 8. Dashed line: fit to eqs 1−3 (see text for details).

values. Apparently, the formation of $(AcO)_2H^-$ in DMSO is insignificant below 0.01 M acetate, but in MeCN the association is significant already in mM range of concentrations of acetate anions. Interestingly, the formation constant for $(AcO)_2H^-$ in MeCN is much higher than that in chloroform, $K_3 = 861\ M^{-1}$.[10]

Thiourea Derivatives 3 and 4. Additions of acetate anions to more acid thiourea derivatives induced deprotonation in both solvents. Spectrophotometric titration of **3** by Bu_4NOAc in DMSO (Figure 2A) shows the appearance of a new band at 482 nm characteristic of deprotonation of the receptor. Indeed, titration with Bu_4NOH produces the same band (Figure 2A, inset), however, of a larger intensity. Another difference is that the titration with acetate anions proceeds with two rather approximate isosbestic points at 380 and 280 nm, but titration with hydroxide involves two different clearly observed isosbestic points at 395 and 289 nm. This comparison indicates that the deprotonation by acetate anions is incomplete and the interaction with acetate may involve an additional product, most probably a hydrogen-bonded complex.

Results of NMR titration, Figure 3A, indeed show characteristics expected for formation of a hydrogen-bonding complex. Signals of NH protons broaden, but the signal of H_1 proton is still detectable and shows a characteristic for hydrogen-bonding strong downfield shift. Signals of aromatic protons also are shifted in the same way as in titration of **1**: H_3 downfield and H_4 upfield. Fitting of the titration profiles to the equation for 1:1 association (Figure 3B illustrates the fitting for protons H_3) gives an approximate value of $K_1 = 1000 \pm 250\ M^{-1}$.

Titration with strong Bu_4NOH base, which definitely leads to deprotonation, shows a completely different pattern: signals of NH protons disappear on addition of ca. 0.1 equiv of hydroxide, and signals of aromatic protons undergo a very small upfield shift by 0.05 ppm and lose their multiplicity in the presence of 1 equiv of hydroxide (Figure 4S, Supporting Information). Also, two new upfield-shifted weak signals appear in the spectrum. Further addition of Bu_4NOH leads to a strong increase in intensity of these new signals, disappearance of initially observed signals, and change of color from orange to deep red probably due to second deprotonation of **3**. Apparently in the presence of 1 equiv of hydroxide both singly and a small amount of doubly deprotonated forms coexist in a slow equilibrium. It seems therefore that the first deprotonation affects very little the positions of aromatic signals apparently because of strong delocalization of the negative charge. As a result of this, the shift in H_3 proton signal induced by a through space interaction with the hydrogen bonded acetate anion becomes a predominant effect.

It is worth noting that while results of spectrophotometric titration clearly show only deprotonation by acetate but do not show any spectral characteristics attributable to hydrogen bonding, the results of NMR titrations on the contrary show only hydrogen bonding interaction without any indication of deprotonation (broadening is often observed during formation of hydrogen-bonded complexes, see e.g., refs 2c and 11). As will be shown below, the reason for this apparent discrepancy is that titration experiments by these two techniques are performed in very different concentration ranges of the receptor.

A possible way to reveal the formation of expected hydrogen-bonded complexes under conditions of spectrophotometric titration is to suppress deprotonation by adding a small amount of acetic acid. Indeed, when the spectrophotometric titration of **3** was performed in the presence of added 1.9 mM AcOH, the spectral course of titration (Figure 2B) corresponded to those expected for the formation of a hydrogen-bonded complex (cf. Figure 1A). Fitting of the titration profiles between 370 and 400 nm to the eq 8 gives after a small correction for the complexation of acetate with added acetic acid the value of K_1 given in Table 1. This value is close to that estimated from the NMR titration (see above) and also is close to K_1 for urea derivative **1**. It should be noted in this connection that more acidic thiourea derivatives do not always form more stable hydrogen-bonded complexes than corresponding urea derivatives. In fact, even formation of less stable complexes with thioureas was reported and attributed to different conformations of thio and oxo derivatives.[12]

Thus, we conclude from above results that the formation of hydrogen-bonded complex between **3** and acetate, reaction 1, and deprotonation of **3**, reaction 2, proceed simultaneously.

Let us consider now the spectrophotometric titration profile for **3** obtained in the absorption maximum of the deprotonated form at 482 nm, Figure 4. The concentration of acetate anions during the titration experiment is always below 1 mM, and therefore, the degree of complexation of acetic acid liberated in the step 2 by acetate anions is always less than 10%, and step 3 can be neglected. Combining equilibria 1 and 2, one obtains for the concentration of deprotonated form the eq 10.

$$[R^-] = [R]_T \frac{(K_2/[AcOH])[AcO^-]}{1 + (K_1 + K_2/[AcOH])[AcO^-]} \qquad (10)$$

Equation 10 predicts a hyperbolic titration curve similar to that for the case of a simple 1:1 complexation process, which, however, should be strongly distorted since concentration of

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acetic acid grows in the course of titration, and therefore, the apparent "binding constant" is variable. Nevertheless, the titration curve fits surprisingly well to eq 8 derived for a simple 1:1 complexation reaction (Figure 4, solid line) with an apparent "binding constant" $K_{app} = (1.41 \pm 0.05) \times 10^4$ M^{-1} 1 order of magnitude larger than K_1. In order to apply physically meaningful eq 10 for the fitting, one needs to resolve mass balance equations for acetate anions and acetic acid and to substitute total concentrations of these components for their free concentrations in eq 10. This leads to too complicated expression and therefore one need to use a more powerful numerical fitting program such as, e.g., Hyperquad, to find the equilibrium constants by iterations. We found it possible however to apply a simplified procedure, which allows one to control better the iteration steps and gives the same final results.

To analyze the titration results in terms of a complete scheme involving all three reactions 1−3, the species distribution diagram was calculated by using the HYSS 2000 (Hyperquad Simulation and Speciation) program[13] with values of K_1 and K_3 determined above and with $K_2 = 1$ as a first approximation. The calculated concentrations of deprotonated form of **3** at variable [Bu_4NOAc] were multiplied by the molar absroptivity of the deprotonated form ($\epsilon_R^- = 1.8 \times 10^4$ M^{-1} cm^{-1}) determined under conditions of complete deprotonation of the receptor by an excess of tetrabutylammonium hydroxide and thus obtained absorbance vs [Bu_4NOAc] profile was compared with the experimentally obtained profile shown in Figure 4. The value of K_2 was varied, initially with a step $\Delta \log K_2 = 0.5$ and then with a step $\Delta \log K_2 = 0.05$ in the range where calculated and experimental profiles were most close to each other, until the best fit shown by a dashed line in Figure 4 was reached. With this manual iteration procedure we obtained $K_2 = 0.050 \pm 0.05$.

The simulation of the titration profile with thus obtained equilibrium constants shows that the "saturation" observed in Figure 4 is to some extent apparent. The fraction of deprotonated form of the receptor reaches 39% at the end of titration (0.8 mM acetate) and further doubling in acetate concentration increases it to only 43%, with what looks like saturation within dispersion of experimental measurements. However, at concentrations of Bu_4NOAc of 10 mM, the equilibrium 3 will be already significant, and further increase in acetate concentration must displace the equilibrium of deprotonation to the right. Surprisingly, we did not observe this, and the degree of deprotonation did not surpass ca. 50% even at concentrations of acetate ions up to 20 mM. A similar effect was also observed in MeCN and will be discussed below.

The species distribution diagram calculated for conditions of the NMR titration experiment (Figure 3) predicts that concentration of the deprotonated form does not exceed 5% of total **3** at highest employed concentration of acetate anions. This explains why the NMR titration shows only formation of the hydrogen-bonded complex. The reason for the low degree of deprotonation is that there is a high concentration of **3** and therefore also a high concentration of acetic acid which would be liberated during significant deprotonation. Note in this connection that the ratio of concentrations of deprotonated and hydrogen bonded forms depends on the absolute concentration of acetic acid (eq 7) rather than on the ratio of concentrations of Bu_4NOAc and receptor.

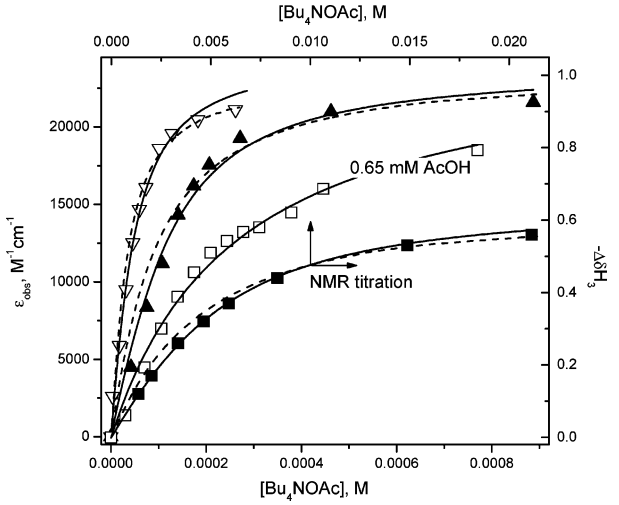

FIGURE 5. Titration curves for receptor **4** (absorbance at 492 nm) with Bu_4NOAc in DMSO at different concentrations: 0.03 mM (open triangles), 0.11 mM (solid triangles), 0.03 mM in the presence of 0.65 mM AcOH (open squares), and 4.4 mM (solid squares, NMR titration, right and upper scales,). Solid lines: formal fits to eq 8. Dashed lines: fits to eqs 2 and 3.

The equilibrium constant for the reaction 2 can be expressed as a ratio of acid dissociation constants of the receptor and acetic acid

$$K_2 = K_a^{RH}/K_a^{AcOH} \tag{11}$$

or in the logarithmic form

$$\log K_2 = pK_a^{AcOH} - pK_a^{RH} \tag{12}$$

From the last relationship, one can estimate $pK_a^{RH} = 13.6$ for **3** from the experimentally measured $\log K_2 = -1.3$ and known $pK_a^{AcOH} = 12.3$ in DMSO.[14] Obviously, **3** is a weaker acid than AcOH; nevertheless, deprotonation by acetate may be nearly quantitative at sufficiently high dilution of the receptor. The pK_a for receptor **1** may be estimated assuming that the effect of substitution of O for S in **3** is equal to the difference in pK_a values for urea (26.9) and thiourea (21) in DMSO.[15] Such an estimate predicts a very high value of $pK_a^{RH} = 19.4$ for **1** and the respective value for $\log K_2 = -7.1$. The calculated degree of deprotonation of 5×10^{-5} M **1** in the presence of 0.01 M acetate anions is only 0.5%. The deprotonation of unsubstituted thiourea by acetate would have $\log K_2 = -8.8$ and cannot be observed even with very high concentration of added acetate anions.

Very weak hydrogen bonding observed with N,N-disubstituted derivative **2** (Table 1) allows one to expect that in DMSO the deprotonation reaction 2 may be the sole process at low acetate concentrations for the receptor **4**. Indeed, the spectral course of titration of **4** by Bu_4NOAc shows the appearance of intense band at 492 nm (Figure 5S, Supporting Information) and is similar to that observed during titration by Bu_4NOH. Titration curves for **4** at two different concentrations of the receptor are shown in Figure 5. Like in the case of **3**, they both can be fitted satisfactorily to the eq 8 with apparent "binding

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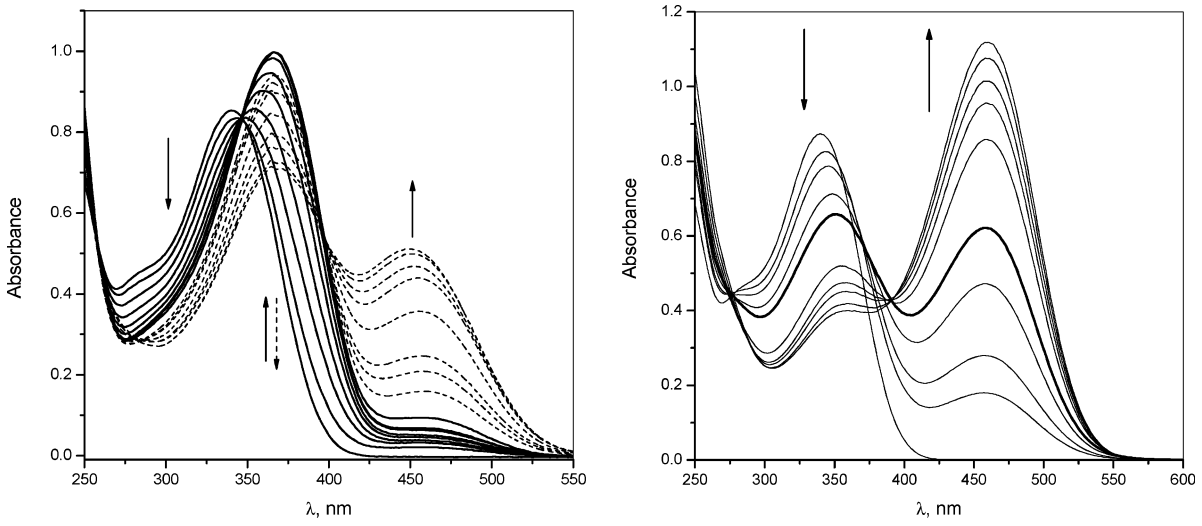

FIGURE 6. (A) Spectrophotometric titration of 6.4×10^{-5} M **3** by Bu_4NOAc in MeCN. Solid lines: spectra recorded at 0−0.5 mM acetate. Dashed lines: spectra recorded at 0.5−25 mM acetate. (B) Titration of 6.4×10^{-5} M **3** by Bu_4NOH (0−0.15 mM) in MeCN. Arrows show the directions of spectral changes at increasing Bu_4NX concentrations.

constants" $K_{app} = (3.6 \pm 0.4) \times 10^4$ M^{-1} for 0.03 mM **4** and $K_{app} = (1.9 \pm 0.3) \times 10^4$ M^{-1} for 0.11 mM **4**. NMR titration of 4.4 mM **4** in DMSO-d_6 shows a pattern typical for deprotonation process (Figure 6S, Supporting Information), and formal fitting of the titration profile for proton H_3 (Figure 5, solid squares) gives $K_{app} = (4.1 \pm 0.2) \times 10^2$ M^{-1}. Thus, all titration profiles can be satisfactorily fitted to the 1:1 binding isotherm, but with "binding constants", which vary by a factor of 100 depending on the concentration of the receptor. At the same time, analysis in terms of eqs 2 and 3 allows one to fit all data with a single value of $K_2 = 2.0 \pm 0.2$ and the value of K_3 given above (the fitting curves are shown as dashed lines in Figure 5). With $K_2 = 2.0$, one obtains from the eq 12 $pK_a^{RH} = 12.0$ for **4**. The reason of higher acidity of **4** as compared to **3** is not clear; probably it is related to some conformational changes induced by second substitution at the nitrogen atom.

Perhaps the most extraordinary aspect of these titration experiments is a very good quality of the fitting to obviously incorrect model of 1:1 complex formation (solid lines in Figures 4 and 5), even surpassing in some cases the fitting quality to experimentally justified deprotonation model (dashed lines in Figures 4 and 5). It follows from the eq 5 that when reaction 2 is the dominant process the expression for K_{app} takes the form

$$K_{app} = [R^-]/[RH][AcO^-] = K_2/[AcOH] \qquad (13)$$

and therefore, K_{app} must vary by a factor of 10 during the titration experiment, which covers an interval of conversion of RH into R^- from ca. 10% to ca. 90%. A possible way to make K_{app} really constant is to perform the titration in the presence of an excess of AcOH over the receptor. The titration plot shown by open squares in Figure 5 was obtained in the presence of 0.65 mM AcOH, 20 times in excess over **4**. As expected, it fits very well to eq 8, and the fitting gives in this case $K_{app} = (3.8 \pm 0.4) \times 10^3$ M^{-1}, reasonably close to the expected value of $K_{app} = 2/[AcOH] = 3.1 \times 10^3$ M^{-1}. Thus, in principle, true values of K_2 for deprotonation may be obtained by simple fitting of titrations performed in the presence of small amounts of added acid to 1:1 binding isotherm and subsequent correction of K_{app} in accordance with eq 13. Surprisingly, we see at the same time that the shape of the titration curve is not significantly affected by a large variation in K_{app}, i.e., K_{assoc} the eq 8. Thus, these apparent "binding constants" may be used for, e.g., comparative purposes, but obviously titrations of different receptors must be performed at the same concentration.

The values of K_{app} obtained in the absence of added acid are proportional to the inverse concentration of **4** in accordance with a simple expression $K_{app} = (1.7 \pm 0.3)/[\mathbf{4}]$. This dependence reflects the fact that the concentration of acetic acid liberated in the system during titration is proportional to total concentration of the receptor. Interestingly, the proportionality coefficient is close to K_2. On the other hand, an attempt to substitute the ratio $K_2/[AcOH]$ in the eq 10 with a constant term $K_2/[\mathbf{3}] = 710$ M^{-1} by analogy with results for **4** fails. By substituting $K_2/[AcOH] = \text{constant} = 710$ M^{-1} together with $K_1 = 1140$ M^{-1} in eq 10, one obtains $K_{app} = 1850$ M^{-1}, a value 7.5-fold lower than that observed experimentally. At the same time, at the end of titration, the ratio $K_2/[AcOH]$ indeed should be close to 700 M^{-1} since according to eq 10 the "saturation" of the titration profile is observed at $[R^-]/[R]_T = (K_2/[AcOH])/(K_1 + K_2/[AcOH])$ and the experimentally observed saturation at 39% of deprotonation requires $K_2/[AcOH] = 730$ M^{-1}. Large observed K_{app} results, therefore, from a very low concentration of acetic acid at the beginning of the titration.

Interaction of **3** with acetate in MeCN also involves both formation of hydrogen-bonded complex and deprotonation, but in this case the reactions are not simultaneous and deprotonation process requires participation of the second acetate anion. The spectral course of the titration in MeCN is shown in Figure 6A. In the range of acetate concentrations 0−0.5 mM, the spectral changes are typical for formation of the hydrogen-bonded complex: the absorption maximum of free receptor at 340 nm is shifted to 366 nm through an isosbestic point at 346 nm, but at higher acetate concentrations a new bond at 452 nm characteristic of the deprotonation process is developed with two isosbestic points at 397 and 284 nm. Titration by Bu_4NOH,

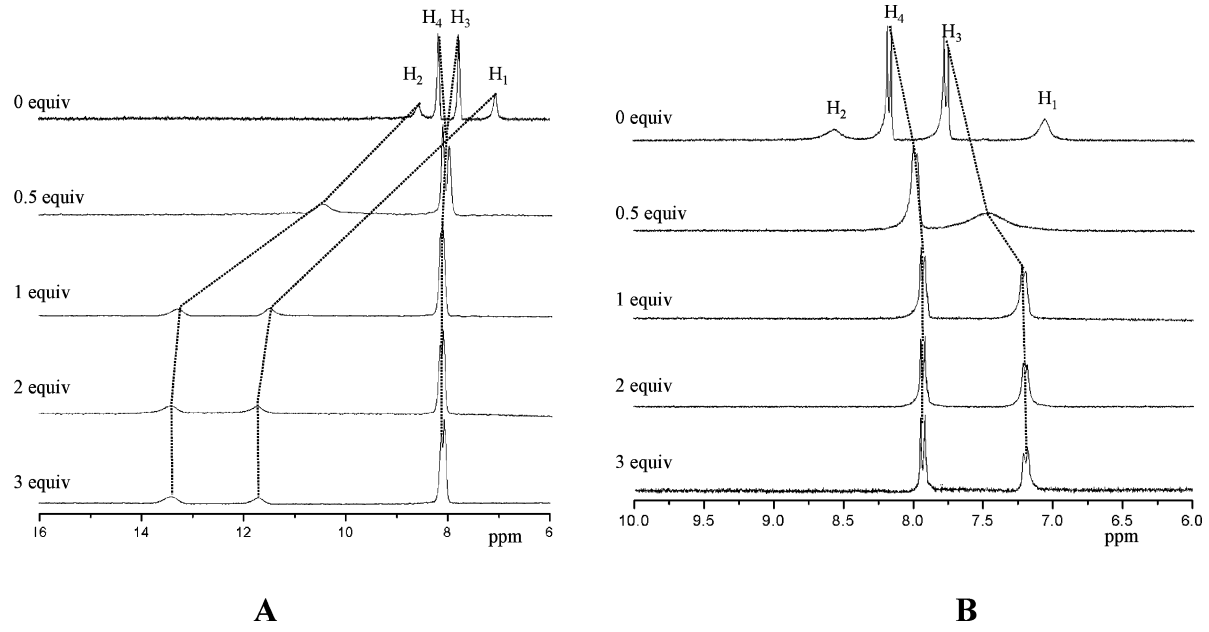

FIGURE 7. Partial ^{1}H NMR spectra of **3** in MeCN-d_3 in the presence of increased amounts of (A) Bu_4NOAc with 9 mM **3** and (B) Bu_4NOH with 15 mM **3**.

Figure 6B, gives a more intense band at 459 nm indicating an incomplete deprotonation of **3** by acetate anions. A shorter wavelength of maximum observed in the presence of acetate ions is due to superimposing of the band of the deprotonated form with the absorption band of the hydrogen-bonded complex. After deconvolution of the final spectrum in Figure 6A the maximum appears at 460 nm. The spectra for titration by hydroxide initially do not pass through an isosbestic point indicating that the reaction with hydroxide may be a more complicated process than just a simple one-step deprotonation.

Results of NMR titration of 9 mM **3** in MeCN by Bu_4NOAc (0−0.075 M) indicate only formation of a 1:1 hydrogen-bonded complex (Figure 7A): signals of H_1 and H_2 are shifted downfield by ca. 4.5 ppm, H_3 also is shifted downfield by 0.2 ppm, and H_4 undergoes a very small upfield shift by 0.05 ppm. There is no indication of deprotonation even in the presence of a 10-fold excess of acetate anions over **3**. Titration with hydroxide shows a typical deprotonation pattern, Figure 7B: signals of NH protons disappear and signals of aromatic protons are shifted upfield. However, the shift of H_4 signal is nearly complete already in the presence of 0.5 mol equivalent of the base and the signal of H_3 undergoes a strong broadening under the same conditions. In spectrophotometric titration, Figure 6B, the spectra start to pass through an isosbestic point after approximately 50% of deprotonation. All these observations indicate that the course of the reaction between **3** and hydroxide is changed after half of the receptor becomes deprotonated. Probably this is due to formation of a hydrogen-bonded complex R_2H^- between anion of **3** and its neutral form analogous to the $(AcO)_2H^-$ complex, which finally becomes completely deprotonated at sufficiently high hydroxide concentration.

Fitting of absorbance vs acetate concentration profiles to the eq 8 at low acetate concentrations give $K_1 = (6 \pm 1) \times 10^4$ M^{-1}. In order to get a more accurate value of K_1, titration of **3** was performed in the presence of added 1.2 mM AcOH, which suppress completely the deprotonation process (Figure 7S, Supporting Information). Fitting of these results gives a smaller observed value of $K_{assoc} = 8400 \pm 500$ M^{-1}, from which after correction for the complexation of acetate with added acetic acid one obtains K_1 value given in Table 1.

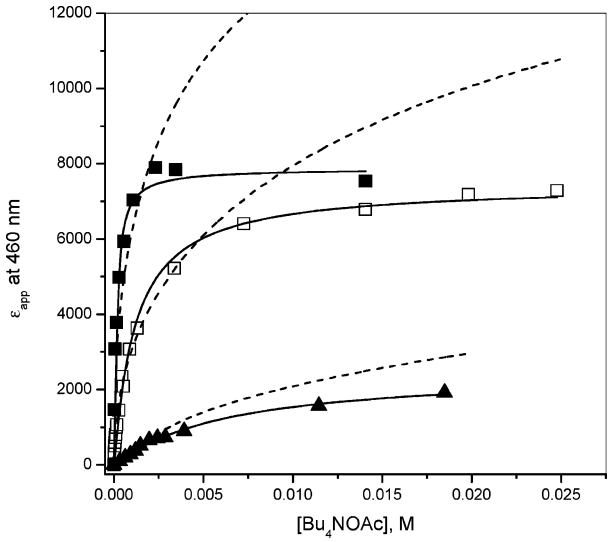

FIGURE 8. Apparent molar absorptivity (ϵ_{app}) of **3** at 460 nm in MeCN vs [Bu_4NOAc] at different concentrations of the receptor: 1.25 mM (solid triangles), 0.064 mM (open squares), 0.013 mM (solid squares). Solid lines are formal fits to the eq 8 and dashed lines are theoretical profiles calculated in accordance with Schemes 1−3 with equilibrium constants given in the text.

The titration profiles at 460 nm obtained at different total concentrations of **3** are shown in Figure 8. They all can be satisfactorily fitted to the eq 8 with formal association constants K_{app} equal to 180 ± 10, 850 ± 70, and 7600 ± 100 M^{-1} for 1.25, 0.064, and 0.013 mM **3**, respectively. The degree of deprotonation at "saturation" also increases on going to more dilute receptor solutions but never reaches 100% (the molar absroptivity of the deprotonated form at 460 nm found from results with hydroxide equals 18700 M^{-1} cm^{-1}). The manual fitting to the scheme involving eqs 1−3 is shown by dashed

lines. Obviously, in all cases the saturation occurs much earlier than predicted theoretically, although results at low acetate concentrations are fitted reasonably well with $K_2 = 0.020 \pm 0.002$ M^{-1}. By using the eq 11 with known pK_a 22.3 of acetic acid in MeCN,[14] one obtains p$K_a = 24.0$ for **3** in this solvent. Thus, we again observe a significant deprotonation of the receptor by acetate anion, which is a base nearly 2 orders of magnitude weaker than anion of **3**.

Deprotonation constants K_2 differ in two solvents very little; just by a factor of 2.5 (see Table 1). Therefore, the fact that in MeCN deprotonation requires the second acetate anion while in DMSO it proceeds simultaneously with formation of the hydrogen-bonded complex is not due to a decreased acidity of the receptor in MeCN. The reason for a different behavior in these solvents is that in MeCN the stability of the hydrogen-bonded complex RHA^- is much higher than in DMSO with K_1 value larger by a factor of 10^2 (Table 1). Therefore, deprotonation via step 2 cannot compete with the complex formation until the concentration of acetate surpasses 0.1 mM, and it starts to remove efficiently acetic acid liberated in step 2 by converting it into a $(AcO)_2H^-$ complex with stability constant K_3 also much larger in MeCN than in DMSO. Thus, less polar MeCN solvent is less favorable for the direct deprotonation because of a higher stability of the hydrogen-bonded complex, but this is compensated by a larger stability of $(AcO)_2H^-$ complex in MeCN as compared with DMSO, and deprotonation becomes possible at higher concentrations of acetate. Such "compensation" is not observed in chloroform, however, where the stability of hydrogen-bonded complexes of anions with urea derivatives is higher than in MeCN (see, e.g., ref 1g) but the stability of $(AcO)_2H^-$ complex is lower (see above). As a result, deprotonation was never reported in this solvent.

The decrease in K_{app} and the yield of the deprotonated form on going to more concentrated receptor solutions is due to higher concentrations of liberated acetic acid, as was the case in DMSO. The simulated species distribution diagram for the conditions of NMR titration predicts the maximum degree of deprotonation less than 10%. This explains why NMR data show only the formation of the hydrogen-bonded complex.

We did not find a satisfactory explanation of the fact that deprotonation by acetate anions is never complete and the titration profiles tend to "saturate" when approximately half of the receptor becomes deprotonated (see Figure 8), even at lowest concentrations of **3**. Probably a part of the problem is mentioned above with possibility of formation of stable R_2H^- species, which may be difficult to further deprotonate by acetate in contrast to much stronger hydroxide base.

Reaction of acetate anions with **4** in MeCN proceeds as a simple complete deprotonation in accordance with both spectrophotometric and NMR titration results. A typical titration profile at the absorption maximum of the deprotonated form is shown in Figure 9, and its formal fit to the eq 8 gives $K_{app} = (3.21 \pm 0.02) \times 10^3$ M^{-1}. The manual fit to a scheme involving reactions 2 and 3 shown by the dashed line gives $K_2 = 0.040 \pm 0.004$ and p$K_a = 23.7$ for **4** in MeCN. As in DMSO, **4** is a stronger acid than **3**, but the effect is much smaller.

Fitting of the results of NMR titration of 12 mM **4** in MeCN gives the same K_2 value and $K_{app} = 60 \pm 7$ M^{-1} (Figure 8S, Supporting Information). The much smaller "binding constant" in this experiment of course is related to higher receptor concentration, although in this case a 50-fold decrease in K_{app} is not inversely proportional to a 220-fold increase in the concentration of the receptor. A smaller than expected decrease in K_{app} may be attributed to higher concentrations of acetate in the NMR titration experiment and, consequently, a stronger shift in the deprotonation equilibrium due to higher degree of complexation of acetic acid into $(AcO)_2H^-$ species.

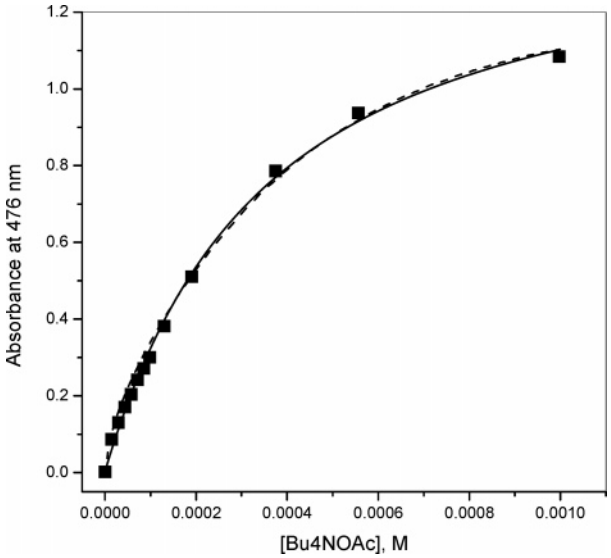

FIGURE 9. Titration curve for 5.5×10^{-5} M **4** (absorbance at 476 nm) with Bu_4NOAc in MeCN. Dashed line: fit to eqs 2 and 3. Solid line − formal fit to eq 8.

Conclusions

The results of this study point to several often overlooked important aspects of interactions between receptors possessing relatively acid proton donor groups and basic anions like acetate, fluoride, or hydrophosphate, capable both to complex them via hydrogen bonding and to deprotonate them. The degree of deprotonation depends on the concentration of the receptor and is larger in more dilute solutions. As a result, an NMR titration performed at high receptor concentration may feel only hydrogen bonding, while a spectrophotometric titration of much more dilute receptor may clearly show the deprotonation. The deprotonation equilibrium can be shifted to a higher yield of the deprotonated receptor by complexation of liberated acid with an excess of anion, but in case of acetate the equilibrium constant for the formation of the complex $(AcO)_2H^-$ is significant only in less polar MeCN solvent. A simple way to measure the equilibrium constant for the formation of the hydrogen-bonded complex is to perform the titration in the presence of added acid, which suppresses the deprotonation process. In order to obtain the equilibrium constant for the deprotonation reaction, a more complicated procedure needs to be employed. We found that a reasonably simple approach consists in a manual iteration procedure employing HYSS program for species distribution calculations. Interestingly, a formal fit to the equation for a 1:1 complexation reaction always is good and allows one to estimate an apparent "binding constant", which, however, lacks any meaning and is a function of total receptor concentration.

Experimental Section

***N*-Methoxyethyl-*N*′-(4-nitrophenyl)urea 1.** To 2-methoxyethylamine (0.50 g, 6.65 mmol) in 20 mL of dry CH_2Cl_2, was added 4-nitrophenyl isocyanate (1 g, 6.65 mmol), and the mixture was

stirred overnight under nitrogen. The solvent was removed under reduced pressure and the residue triturated sequentially with MeOH and hexane. The product was collected by filtration to give **1** (1.13 g) as a pale yellow powder in 71% yield: mp 141−143 °C; IR (KBr) 3330, 1644 cm^{-1}; ^1H NMR (DMSO-d_6) δ_{DMSO} 3.40 (t, J = 5.56 Hz, 2H), 6.59 (s, 1H), 7.61 (d, J = 8.14 Hz, 2H), 8.14 (d, J = 8.11 Hz, 2H), 9.32 (s, 1H); lkyl protons are overlapping with the residual water protons of the solvent; ^{13}C NMR (DMSO-d_6) δ 57.92, 70.94, 116.76, 125,16, 140.41, 147.10, 154.37; MS(FAB, m/z) 240 (M^+). Anal. Calcd for $C_{10}H_{13}N_3O_4$ (239.23): C, 50.21; H, 5.48; N, 17.56. Found: C, 50.20; H,5.48; N,17.55.

***N*-Methoxyethyl-*N′*-(4-nitrophenyl)thiourea 3.** A procedure similar to that used for **1** was applied with a yield of 75%: mp 121−123 °C; IR (KBr) 3296, 3198, 1308 cm^{-1}; ^1H NMR (300 MHz DMSO-d_6) δ_{DMSO} 3.50 (t, J = 5.19 Hz 2H), 3.68 (br s, 2H), 7.87 (d, J = 9.16 Hz, 2H), 8.18 (d, J = 9.17 Hz, 2H), 8.27 (br s, 1H), 10.22 (br s, 1H); alkyl protons are overlapping with the residual water protons of the solvent; ^{13}C NMR (DMSO-d_6) 43.53, 58.00, 69.67, 120.31, 124.51, 141.80, 146.39, 180.09; MS(FAB, m/z) 256 (M^+). Anal. Calcd for $C_{10}H_{13}N_3O_3S$ (255.29): C, 47.05; H, 5.13; N, 16.46. Found: C, 47.09; H, 5.12; N, 16.47.

***N*,*N*-Bis(methoxyethyl)-*N′*-(4-nitrophenyl)urea 2:** mp 109−111 °C; IR (KBr) 3322, 1654 cm^{-1}; ^1H NMR (DMSO-d_6) δ_{DMSO} 3.52 (dt, J = 21.75, 4.85 Hz, 2H), 7.66 (d, J = 9.36 Hz, 2H), 8.11 (d, J = 9.31 Hz, 2H), 9.00 (s, 1H); alkyl protons are overlapping with the residual water protons of the solvent; ^{13}C NMR (DMSO-d_6) δ 47.17, 58.30, 70.72, 118.30, 124.77, 140.83, 124.24, 154.54; MS (FAB, m/z) 298 (M^+). Anal. Calcd for $C_{13}H_{19}N_3O_5$ (297.31): C, 52.52; H, 6.44; N, 14.13. Found: C, 52.55; H,6.43; N,14.13.

***N*,*N*-Bis(methoxyethyl)-*N′*-(4-nitrophenyl)thiourea 4:** mp 123−125 °C; IR (KBr) 3436, 3268, 1322 cm^{-1}; ^1H NMR(DMSO-d_6) δ_{DMSO} 3.64 (t, J = 5.19 Hz, 2H), 3.98 (br s, 2H), 7.63 (d, J = 9.28 Hz, 2H), 8.17 (d, J = 9.23 Hz, 2H), 9.69 (s, 1H); alkyl protons are overlapping with the residual water protons of the solvent; ^{13}C NMR (DMSO-d_6) δ 52.38, 59.15, 70.79, 123.48, 124.64, 142.93, 147.27, 181.10; MS (FAB, m/z) 314 (M^+); Anal. Calcd for $C_{13}H_{19}N_3O_4S$ (313.37): C, 49.83; H, 6.11; N, 13.41. Found: C, 49.81; H, 6.12; N, 13.43.

Spectrophotometric and ^1H NMR Titrations. The absorption spectra were recorded after additions of aliquots of Bu_4NOAc or Bu_4NOH stock solutions in respective solvents to a 10^{-5}−10^{-4} M receptor solution in DMSO or MeCN in a quartz cuvette placed in a compartment of a diode array spectrophotometer thermostated at 25 ± 0.1 °C with a recirculating water bath. NMR titrations were performed on a 300 MHz spectrometer with more concentrated stock solutions in the respective deuterated solvents adding aliquots of them to 5−20 mM receptor solutions in DMSO-d_6 or CH_3CN-d_3 directly to NMR tubes. In order to test possible autoassociation of receptors **1**−**4** their NMR spectra were recorded in a range of concentrations from 3 to 25 mM in both solvents and signals of all protons were found unchangeable. Nonlinear least-squares fits of the experimental results to the eq 8 or its analogue for NMR titration were performed by using the Microcal Origin version 5 program.

Acknowledgment. Carol Pérez-Casas thanks DGAPA UNAM for the postdoctoral fellowship.

Supporting Information Available: General experimental methods; ^1H and ^{13}C NMR spectra for obtained compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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