Anion Binding in Aqueous Solutions by *N*-(Isonicotinamido)-*N*′-phenylthiourea-Based Simple Synthetic Neutral Receptors. Role of the Hydrophobic Microenvironment of the Receptor Molecule

Rui Yang,[†,‡] Wen-Xia Liu,[†] Hong Shen,[†] Hui-Hui Huang,[†] and Yun-Bao Jiang*[,†]

Department of Chemistry, College of Chemistry and Chemical Engineering, and The MOE Key Laboratory of Analytical Sciences, Xiamen University, Xiamen 361005, China, and College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China

Received: December 19, 2007; In Final Form: February 13, 2008

N-(Isonicotinamido)-*N*′-(substituted-phenyl)thioureas (**1a**−**e**, substituent X = *p*-OCH_3, *p*-CH_3, H, *m*-Br, and *m*-CF_3) have been designed as neutral receptors, in order to prove the influence of conformational issues on the ability to bind anions in aqueous solutions. Compounds **1a**−**e** were shown to create a hydrophobic microenvironment around the thiourea group, favoring hydrogen bonding interactions, by evidence from quantum mechanic calculations, thermodynamic analysis, NMR aromatic current shielding, and comparative anion binding. Referring to *N*-(substituted-benzamido)thioureas (**2a**−**e**, substituent Y = H, *m*-Cl, *m*-NO_2, *m*,*m*-Cl,Cl, and *p*-NO_2), we showed that, for the hydrophobic microenvironment to be operative in aqueous solutions, the amido −NH proton needs to be acidic enough.

1. Introduction

Recognition and sensing of anions by synthetic receptors have seen substantial recent progress thanks to the understanding of supramolecular chemistry of anions, together with the employment of sophisticated synthetic chemistry.[1−3] Anion recognition is, in general, made possible via hydrogen bonding and/or electrostatic interactions. Both of the two interactions become weaker in aqueous solutions, with anion sensing by simple neutral receptors via hydrogen bonding suffering more. In order to make anion sensing possible in aqueous solutions, structural modifications on the receptor molecule by incorporating more binding sites in a suitable arrangement has been successful to varied extents, demanding synthetic efforts. With neutral receptors themselves, increasing the acidity of the hydrogen bonding donors has also been a strategy of first choice. That, however, may lead to deprotonation of the highly acidic hydrogen bonding donor in the presence of alkaline anion in aqueous solutions.[4−6]

It is a bit surprising that the possible role of the receptor molecular conformation in terms of providing a good microenvironment for anion binding has not been paid much attention.[7−9] On the basis of solvent dependence of hydrogen bonding, it appears that, in general, a less polar medium is favorable.[10] We thus envisaged that by providing a less polar microenvironment for the binding site, efficient hydrogen bonding might be allowed in aqueous solutions. With neutral receptors, this will be possible if the binding site is made between two hydrophobic groups. The two terminals will then approach as a result of, for example, hydrophobic interaction in aqueous solutions,[11,12] leading to a cleft with a less polar microenvironment. We accordingly designed a series of simple neutral receptors, *N*-(isonicotinamido)-*N*′-(substituted-phenyl)thioureas (substituent X = *p*-OCH_3, *p*-CH_3, H, *m*-Br, and *m*-CF_3, **1a**−**e**, Figure 1), which have two aryl terminals. A pyridine terminal was chosen in order to also improve the water solubility and prevent self-aggregation of the receptor molecules in aqueous solutions. An *N*-amidothiourea binding site was employed following our previous work that showed a substantially enhanced anion binding affinity in the organic solvent acetonitrile (MeCN).[13−15] Quantum mechanic calculations at the B3LYP/6-31G* level[16] indeed suggested that the two aryl rings in **1c** could approach to within a van der Waals contact, thereby affording a hydrophobic microenvironment. This was supported by a variety of other evidence. Results do show that the absorption spectra of **1a**−**e** could sensitively respond to anions such as AcO^- in aqueous solution with binding constants of 10^3 M^{-1} order of magnitude that are comparable to or even higher than those of the classical *N*,*N*′-diphenylthioureas in an noncompetitive solvent such as MeCN.

2. Experimental

^1H NMR and ^{13}C NMR were acquired in DMSO-d_6 or CD_3CN on a Bruker AV400 MHz or Varian Unity$^+$ 500 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard. High-resolution mass spectrometry (HRMS) spectra were obtained on a Micromass LCT spectrometer by injection of the methanol solution of the sample. Absorption spectra were recorded on a Varian Cary 300 absorption spectrophotometer. Anions used for binding titrations in organic solvents were their *n*-Bu_4N^+ salts commercially available. Inorganic salts used in this research were those of the highest purity available in the market. The solvents employed for titrations were redistilled MeCN and deionized water.

1a−**e** were synthesized by reacting *N*-(isonicotinyol)hydrazine with phenyl isothiocyanates in ethanol at room temperature for 2 h. **2a**−**c** and **2e** were those available in the laboratory, and **2d** was prepared following the same procedures.[14] Stirring phenylisothiocyanate (2 mmol) with hydrazine monohydrate

* Corresponding author. Phone/Fax: +86 592 2185662. E-mail: ybjiang@xmu.edu.cn.
† Xiamen University.
‡ Southwest University.

10.1021/jp711899h CCC: $40.75 © 2008 American Chemical Society
Published on Web 04/03/2008

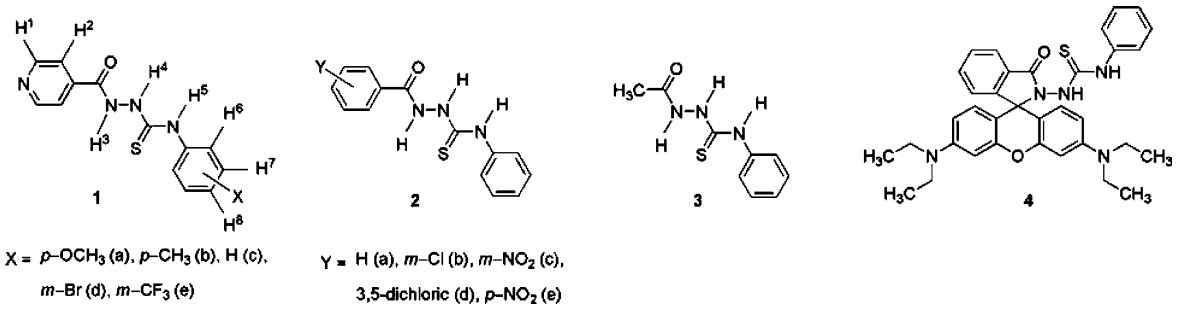

Figure 1. Structures of **1a**−**e** and model molecules **2**−**4**. Numbering of protons is given for **1**.

(80%, 6 mmol) in ethanol at room temperature for 2 h produced *N*-amino-*N*′-phenylthiourea (1.5 mmol), which, after reaction with acetic anhydride (1.5 mmol) in acetic acid (3 mL), under stirring at room temperature for 1h, afforded **3**. The lactam control molecule **4** was synthesized following a reported procedure.[17] The as-obtained products were purified by repeated recrystallizations from ethanol. All newly synthesized molecules were fully characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. **1a**: ^{1}HNMR (400 MHz, DMSO-d_6), δ (ppm) 10.82 (s, 1H), 9.75 (s, 1H), 9.70 (s, 1H), 8.77 (d, 2H, J = 6.4 Hz), 7.85 (d, 2H, J = 5.6 Hz), 7.27 (d, 2H, J = 8.4 Hz), 6.90 (d, 2H, J = 8.8 Hz), 3.75 (s, 3H); ^{13}CNMR (100 MHz, DMSO-d_6), δ (ppm) 181.5, 164.7, 157.0, 150.2, 139.7, 132.0, 127.7, 121.8, 113.4, 55.3; HRMS (ESI) calcd for $C_{14}H_{14}N_4O_2S$ 303.0916 (M + H$^+$), found 303.0910 (M + H$^+$). **1b**: ^{1}HNMR (400 MHz, DMSO-d_6), δ (ppm) 10.83 (s, 1H), 9.80 (s, 1H), 9.74 (s, 1H), 8.77 (d, 2H, J = 6.0 Hz), 7.85 (d, 2H, J = 7.5 Hz), 7.28 (d, 2H, J = 7.2 Hz), 7.14 (d, 2H, J = 8.4 Hz), 2.28 (s, 3H); ^{13}CNMR(100 MHz, DMSO-d_6), δ (ppm) 181.3, 164.6, 150.2, 139.7, 136.6, 134.5, 128.6, 126.0, 121.8, 20.6; HRMS (ESI) calcd for $C_{14}H_{14}N_4OS$ 287.0967 (M + H$^+$), found 287.0966 (M + H$^+$). **1c**: ^{1}HNMR (400 MHz, DMSO-d_6), δ (ppm) 10.86 (s, 1H), 9.87 (s, 1H), 9.82 (s, 1H), 8.78 (d, 2H, J = 4.0 Hz), 7.86 (d, 2H, J = 3.6 Hz), 7.43 (s, 2H), 7.34 (t, 2H, J = 6.2 Hz), 7.17 (t, 1H, J = 5.8 Hz); ^{13}CNMR (100 MHz, DMSO-d_6), δ (ppm) 181.1, 164.52, 150.2, 139.6, 139.1, 128.1, 126.1, 125.2, 121.7; HRMS (ESI) calcd for $C_{13}H_{12}N_4OS$ 273.0810 (M + H$^+$), found 273.0807 (M + H$^+$). **1d**: ^{1}HNMR (400 MHz, DMSO-d_6), δ (ppm) 10.89 (s, 1H), 9.98 (s, 1H), 9.90 (s, 1H), 8.78 (d, 2H, J = 4.8 Hz), 7.85 (d, 2H, J = 4.8 Hz), 7.70 (s, 1H), 7.50 (t, 1H, J = 7.6 Hz), 7.36 (d, 1H, J = 8.0 Hz), 7.30 (t, 1H, J = 7.8 Hz); ^{13}CNMR (100 MHz, DMSO-d_6), δ (ppm) 181.0, 164.5, 150.2, 140.8, 139.5, 129.9, 128.1, 127.7, 124.6, 121.7, 120.4; HRMS (ESI) calcd for $C_{13}H_{11}BrN_4OS$ 350.9915 (M + H$^+$), found 350.9918 (M + H$^+$). **1e**: ^{1}HNMR (400 MHz, DMSO-d_6), δ (ppm) 10.92 (s, 1H), 10.06 (s, 1H), 10.02 (s, 1H), 8.79 (d, 2H, J = 6.0 Hz), 7.87−7.81 (m, 4H), 7.58 (t, 1H, J = 7.8 Hz), 7.52 (d, 1H, J = 8.0 Hz); ^{13}CNMR (100 MHz, DMSO-d_6), δ (ppm) 181.19, 164.5, 150.3, 140.0, 139.4, 129.6, 129.2, 128.9, 128.6, 125.3, 122.7, 121.7; HRMS (ESI) calcd for $C_{14}H_{11}F_3N_4OS$ 341.0684 (M + H$^+$), found 341.0679 (M + H$^+$). **2d**: ^{1}HNMR (400 MHz, DMSO-d_6), δ (ppm) 10.79 (s, 1H), 9.84 (s, 1H), 9.79 (s, 1H), 7.95 (s, 2H), 7.89 (s, 1H), 7.40 (s, 2H), 7.34 (t, 2H, J = 7.6 Hz), 7.18 (t, 1H, J = 7.2 Hz); ^{13}CNMR (100 MHz, DMSO-d_6), δ (ppm) 181.1, 163.6, 139.1, 135.8, 134.3, 131.2, 128.1, 126.8, 126.3, 125.4; ESI-MS calcd for $C_{14}H_{11}Cl_2N_3O_S$ 341.2 (M + H$^+$), found 341.2 (M + H$^+$). **3**: ^{1}HNMR (400 MHz, DMSO-d_6), δ (ppm) 9.86 (s, 1H), 9.60 (s, 1H), 9.51 (s, 1H), 7.42 (d, 2H, J = 7.6 Hz), 7.33 (t, 2H, J = 7.2 Hz), 7.16 (t, 1H, J = 7.2 Hz), 1.89 (s, 3H); ^{13}CNMR (100 MHz, DMSO-d_6), δ (ppm) 181.0, 169.2, 139.2, 128.0, 125.8, 125.1, 21.0; HRMS (ESI) calcd for $C_9H_{11}N_3OS$ 210.0701 (M+H$^+$), found 210.0703 (M + H$^+$). **4**: ^{1}H NMR (400 MHz, DMSO-d_6), δ (ppm) 9.38 (s, 1H), 8.81 (s, 1H), 7.90 (d, 1H, J = 6.8 Hz), 7.66−7.58 (m, 2H), 7.17 (t, 2H, J = 7.6 Hz), 7.12−7.05 (m, 5H), 6.36 (s, 2H), 6.28 (s, 3H), 3.32−3.29 (m, 8H), 1.07 (t, 12H, J = 6.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm) 181.2, 166.3, 153.5, 150.8, 148.4, 138.7, 133.7, 129.5, 128.7, 127.6, 125.6, 125.6, 125.1, 124.2, 122.9, 107.9, 104.4, 97.0, 66.2, 43.7, 12.5; HRMS (ESI) for $C_{35}H_{37}N_5O_2S$ calcd 592.2746 (M + H$^+$), found 592.2754 (M + H$^+$).

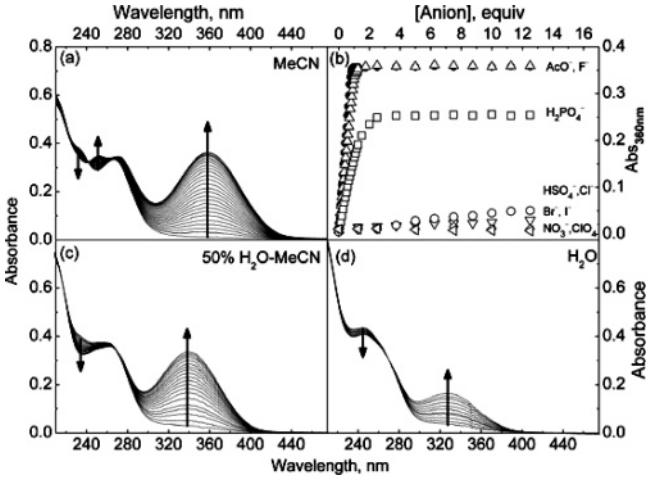

Figure 2. Absorption spectra of **1c** in the presence of AcO$^-$ in MeCN (a), 50% H_2O−MeCN (c), and H_2O (d), and plots of absorbance of the new band at 360 nm against anion concentration (b). Anions existed in their *n*-Bu_4N^+ salts (a, b) and in NH_4OAc for AcO$^-$ (c, d). The aqueous solution pH of 5.5 was tuned by using NaOH/HCl; here Cl$^-$ was considered not to bind to **1c** in aqueous solutions since even in MeCN no appreciable binding was observed (see Figure 1b). [**1c**] = 20 μM.

3. Results

3.1. Absorption Spectra. Anion binding of **1a**−**e** was first examined in MeCN. **1a**−**e** exhibited in MeCN an absorption band at ca. 267 nm. Upon introducing AcO$^-$, F$^-$, and $H_2PO_4^-$ anions, a new and substantially red-shifted band at ca. 360 nm was developed, together with a clear isosbestic point at ca. 240 nm. Figure 2a shows the absorption titration traces of **1c** by AcO$^-$. Spectral data for other members of **1** can be found in Table 1. The appearance of a clear isosbestic point suggests the formation of well-defined complexes between **1a**−**e** and the tested anions. Job plot analysis for **1c**/AcO$^-$ interaction in MeCN probed a 1:1 binding stoichiometry. The addition of other anions such as ClO_4^-, NO_3^-, HSO_4^-, Cl$^-$, Br$^-$, and I$^-$ induced much less or no change in the absorption spectra (see, for example, in Figure 2b the absorbance at 360 nm of **1c** as a function of anion concentration). No such spectral changes were

TABLE 1: Absorption Spectral Parameters of Receptors 1a−e and Receptor−Anion Binding Complexes, and Anion Binding Constants in MeCN[a]

				K_a, M^{-1} [b]		
R	λ_R^{max}, nm[c]	$\lambda_{R+AcO^-}^{max}$, nm[d]	$\Delta h\nu$ cm^{-1} [e]	AcO^-	F^-	$H_2PO_4^-$
1a	263	358	10090	$> 10^7$ [f]	$(2.08 \pm 0.40) \times 10^6$	$(1.22 \pm 0.27) \times 10^5$
1b	265	359	9881	$> 10^7$ [f]	$(6.47 \pm 0.20) \times 10^6$	$(5.59 \pm 0.14) \times 10^5$
1c	267	360	9675	$> 10^7$ [f]	$(6.63 \pm 0.60) \times 10^6$	$(7.90 \pm 0.80) \times 10^5$
1d	270	360	9259	$> 10^7$ [f]	$> 10^7$ [f]	$(1.65 \pm 0.36) \times 10^6$
1e	271	361	9199	$> 10^7$ [f]	$> 10^7$ [f]	$(3.94 \pm 0.84) \times 10^6$

[a] Anions existed in their *n*-Bu_4N^+ salts. [b] Binding constants of ClO_4^-, NO_3^-, HSO_4^-, Cl^-, Br^-, and I^- were not available because of the minor spectral change. [c] Absorption maximum of receptor. [d] Absorption maximum of receptor-AcO^- complex. [e] AcO^- binding induced red shift in absorption maximum. [f] Binding constant is too high to allow for a precise evaluation.

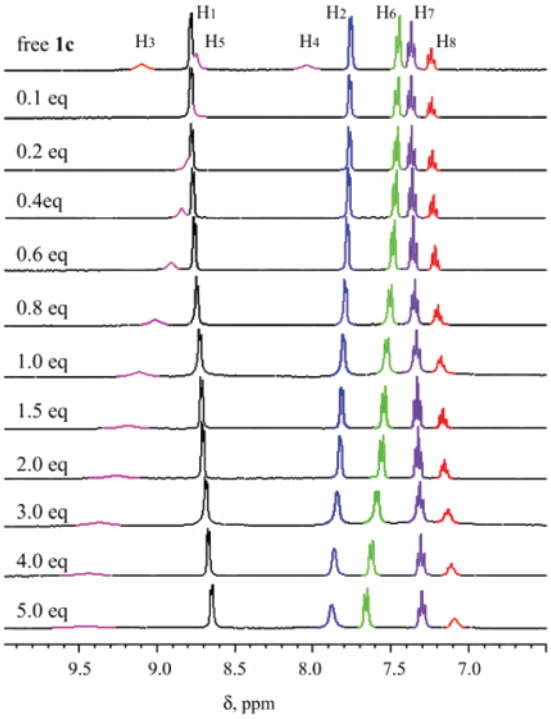

Figure 3. ^1H NMR traces of titration by AcO^- of **1c** in CD_3CN. For numbering of protons, see Figure 1. [**1c**] = 10 mM.

found in control experiments using *N*-(isonicotinyol)hydrazine, a thiourea-absent analogue of **1c**, suggesting that the spectral changes observed for **1c** resulted from anion hydrogen bonding to its thiourea moiety.[1−3,13−15] Note that the new absorption band of **1a−e** in the presence of anions in MeCN located at ca. 360 nm could similarly be assigned to a charge transfer (CT) absorption as done with *N*-benzamidothiourea counterparts **2**.[14] The CT absorption of the anion complexes of **1** located at longer wavelength than that of **2** of 330−350 nm, suggesting a higher electron withdrawing ability of the *N*-pyridine ring in **1** compared to the *N*-phenyl ring in the reported members of **2**.[14]

3.2. ^1H NMR Titrations. Hydrogen-bonding nature of the anion-receptor interaction was confirmed by ^1H NMR titrations of **1c** in CD_3CN by AcO^- (Figure 3). Signals of the thioureido NH protons in **1c** appeared at 8.74 and 8.04 ppm and of the amido NH proton at 9.10 ppm, respectively. Addition of AcO^- resulted in obvious changes in the thioureido NH signals. Whereas it was hard to locate the H^4 signal, the peak area integral data suggested that the initial two thioureido NH signals probably merged into a single one, which then underwent downfield shift to give a broadened and weakened signal at 9.47 ppm at 5 equiv of AcO^-. The amido NH signal was too broad to be followed. The dramatic downfield shift of the thioureido NH signals suggested the hydrogen bonding of these NHs with AcO^-. Meanwhile, it was observed that the aromatic C−H2 and C−H6 protons next to the hydrogen-bonding site underwent a downfield shift, whereas C−H1, C−H7, and C−H8 protons that are a bit away experienced a moderate upfield shift, a profile indicative of the hydrogen-bonding nature of the receptor−anion interaction.[18]

3.3. Anion Binding Constant. Binding constants of **1a−e** with anions in MeCN were evaluated by nonlinearly fitting[19] the absorbance of the new absorption band against anion concentration, following a 1:1 binding stoichiometry that was probed from Job plots (data not shown). From the data given in Table 1, it is noted that the binding constants of **1a−e** with AcO^- are over 10^7 M^{-1} orders of magnitude, which are much higher than those of the classical *N*,*N*′-diphenylthioureas of 10^2 to 10^3 M^{-1}. The high anion binding constants of **1a−e** in MeCN are characteristic of *N*-(benzamido)thioureas.[13−15] Although the extremely high binding constants of AcO^- and F^- do not allow for a credible correlation with the Hammett constant of the substituent X (σ_X) at the *N*′-phenyl ring in **1a−e**, those of $H_2PO_4^-$ do correlate linearly to σ_X by $\ln K_a$ ($H_2PO_4^-$) = 13.3 + 3.74σ_X (n = 5, R = 0.9221). The linear slope is higher than that of *N*-phenylthioureas with AcO^- of 2.52,[15] again indicating an amplification of the substituent effect on anion binding as previously observed with *N*-benzamidothioureas (**2**, Figure 1).

3.4. Anion Binding in Aqueous Solutions. Spectral titrations by anions of **1c** were then carried out in H_2O−MeCN of varied water content. In 50% H_2O−MeCN of pH 5.5 for example, a broad absorption band was observed at 263 nm with a shoulder at 340 nm (Figure 2c). Upon incremental addition of AcO^-, absorbance at 340 nm increases at the expense of the 263 nm band together with the appearance of an isosbestic point at 270 nm. Other anions such as F^-, $H_2PO_4^-$, and Cl^- were also investigated. NaF and KH_2PO_4 lead to spectral changes similar to NH_4OAc, whereas NH_4Cl produces essentially no variation. The solution pH was found to change by less than 0.3 units during AcO^- titrations, indicating that the absorption change did not result from pH variation. Spectral response was also found at other water contents up to pure water solution and detailed data for the absorption spectral parameters of **1c** and **1c**−AcO^- complexes, and **1c**−AcO^- binding constants in H_2O−MeCN of varied composition can be found in Table 2. The **1c**−AcO^- binding constant indeed decreases gradually with increasing water percentage, yet it is still as high as 10^3 M^{-1} orders of magnitude in pure water that actually ensures a sensitive spectral response toward AcO^- (Figure 2d). Meanwhile, the new CT absorption band shifts to the blue. We found that both the energy (eV) of the CT absorption at 329−360 nm and the logarithm of AcO^- binding constant varied linearly with water volume fraction by equations of $h\nu_{CT}$ (abs) = 3.55 + 0.215 [H_2O] ([H_2O] > 0, n = 9, R = 0.9959) and $\ln K_a$ = 12.14 − 5.33 [H_2O] (n = 10, R = 0.9875), respectively. With

TABLE 2: Absorption Spectral Parameters of 1c and 1c−AcO⁻ Complex, and 1c−AcO⁻ Binding Constant in pH 5.5 H_2O−MeCN of Varied Water Molar Fraction f_{H_2O}[a]

H_2O, v/v	f_{H_2O}	λ_R^{max}, nm[b]	$\lambda_{R+AcO^-}^{max}$, nm[c]	$\Delta h\nu$, cm^{-1} [d]	K_a (AcO^-), M^{-1}	ΔG, kJ mol^{-1} (298K)
0	0	267	360	9675	$>10^7$	
0.1	0.24	265	347	8917	$(1.66 \pm 0.14) \times 10^5$	−29.8
0.2	0.42	264	345	8893	$(5.11 \pm 0.16) \times 10^4$	−26.9
0.3	0.55	264	343	8724	$(3.88 \pm 0.35) \times 10^4$	−26.2
0.4	0.66	264	341	8553	$(1.79 \pm 0.09) \times 10^4$	−24.3
0.5	0.74	263	340	8611	$(1.30 \pm 0.06) \times 10^4$	−23.5
0.6	0.81	260	338	8876	$(8.80 \pm 0.43) \times 10^3$	−22.5
0.7	0.87	258	336	8998	$(2.73 \pm 0.29) \times 10^3$	−19.6
0.8	0.92	254	333	9340	$(2.80 \pm 0.18) \times 10^3$	−19.7
0.9	0.96	250	331	9788	$(1.75 \pm 0.11) \times 10^3$	−18.5
1.0	1.00	245	329	10421	$(1.11 \pm 0.14) \times 10^3$	−17.4

[a] AcO^- existed as its NH_4^+ salt. [b] Absorption maximum of receptor. [c] Absorption maximum of receptor−AcO^- binding complex. [d] AcO^- binding induced red shift in absorption maximum.

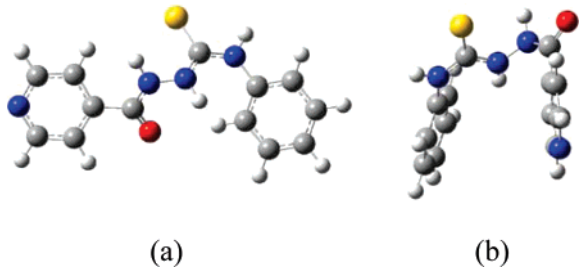

Figure 4. B3LYP/6-31G* optimized separate (a) and contractive (b) conformations of **1c**.

other members of **1**, a sensitive spectral variation was similarly observed (see data given later in 70% and 90% H_2O−MeCN solutions).

4. Discussion

Hydrogen bonding in general becomes weaker upon increasing solvent polarity, in the order of CCl_4 > $CHCl_3$ > CH_3CN > DMSO > CH_3OH > H_2O.[10,20] Anion affinity of a hydrogen-bonding-based neutral receptor is thus low in the highly polar aqueous solutions. Weak hydrogen bonding in a polar solvent has been ascribed to strong anion solvation that lowers enthalpy gain from anion−receptor interaction by the enthalpy cost of anion desolvation.[10] The conformation of the receptor molecule itself in highly polar solvent water, however, has not been paid much attention.[7−9] Examining the molecular structure of **1**, it appeared that a less polar microenvironment afforded by the approach of two aromatic terminals in polar solvents driven by hydrophobic interaction[10,20] likely contributed to their highly efficient anion binding in aqueous solutions.

4.1. Evidence for Hydrophobic Microenvironment Afforded by 1. Gas-phase molecular mechanics calculations at the B3LYP/6-31G* level[16] indicated that **1c** assumed two stable conformations: separate and contractive (Figure 4). Despite a lower calculated energy for the separate conformation in gas phase by 5.11 kcal mol^{-1} than that of the contractive conformation, the calculated dipole moment (7.23 D) of the contractive conformation is much higher than 4.55 D of the separate conformation. The contractive form was therefore expected to be stabilized to a higher extent in polar solvents. The cleft thus formed between two approaching aryl rings is obviously hydrophobic, a microenvironment that is good for its hydrogen bonding with anions.

Water-content dependence of the anion binding constant[12] supported this assumption. It is known that small anions such as AcO^- are better solvated in water. Hefter et al.[21] investigated the thermodynamics of AcO^- transferring from H_2O to H_2O−MeCN mixtures. On the basis of the available data from this investigation,[21] it was deduced that, over an f_{H2O} range of 0.6−0.95, desolvation of AcO^- became 3.6 kJ mol^{-1} more costly in free energy for every 10% increment in f_{H2O}. Obviously, if only the increased cost of anion desolvation was operative, the AcO^- binding constant of **1c** would assume a 160-fold drop upon increasing f_{H2O} from 0.60 to 0.95. Experiments, however, showed that the binding constant decreased only by 10-fold from 1.79×10^4 to 1.75×10^3 M^{-1} (Table 2). We thus assumed that the presence of a hydrophobic microenvironment that is good for anion binding compensated to some extent the free energy cost in AcO^- desolvation.

In order to obtain further information on receptor conformation, the *N*-aromatic ring current effect on the chemical shifts of *N*′-phenyl CH protons in **1c** and **2a** was monitored in D_2O−CD_3CN of increasing D_2O content. This was carried out by comparing changes in the chemical shifts with those in an *N*-acetamidothiourea control molecule (**3**, Figure 1) that has no such *N*-aromatic ring current effect. The solvent-polarity dependent changes in the chemical shifts of the *N*′-phenyl CH protons in **1c** and **2a** can be corrected by using those in **3**, which bears no *N*-phenyl ring. If the two aryl rings in **1c** and **2a** approach in polar solvents, the chemical shifts of the *N*′-phenyl aromatic protons would be affected by the ring current of the *N*-aromatic ring, differing from the case in **3**, whose aromatic protons' chemical shifts are only affected by the solvent polarity change. The differences in the chemical shifts of the *N*′-phenyl aromatic protons ($\Delta\delta$) of **1c** and **2a** from those of **3**, respectively, are listed in Table 3. It is noted that the chemical shifts of *ortho*-H (*o*-H), *meta*-H (*m*-H), and *para*-H (*p*-H) at the *N*′-phenyl ring of **1c** and **2a** indeed undergo gradual upfield shifts with increasing D_2O content. These differences support the approach of the two aryl rings in **1c** and **2a** in polar solvents, which induces an increment of the shielding effect on *N*′-phenyl protons by the *N*-aromatic ring current.[22]

Variations of the binding constants of **1c** with anions of varying hydrophobicity in H_2O−MeCN of increasing water content also suggest the contribution of a hydrophobic cleft assumed by the two aryl rings in **1c**. On the basis of the binding constants of benzoate ($C_6H_5CO_2^-$), acetate ($CH_3CO_2^-$), and propionate ($CH_3CH_2CO_2^-$) given in Table 4, it is clear that, at lower water content, the binding constant of $C_6H_5CO_2^-$ is smaller than that of $CH_3CO_2^-$ because of a lower electron density in $C_6H_5CO_2^-$. Upon increasing water content, however, the binding constant of $C_6H_5CO_2^-$ bearing a more hydrophobic phenyl ring becomes close to that of $CH_3CO_2^-$ and even approaches that of $CH_3CH_2CO_2^-$, whose anion binding constant is higher than that of $CH_3CO_2^-$ in a more polar medium because of a longer alkyl chain that has a stronger hydrophobic interaction with the less polar microenvironment afforded by the receptor molecule. Obviously, the apparent rate of anion binding constants of **1c** decreasing with water content in the order of $C_6H_5CO_2^-$ < $CH_3CH_2CO_2^-$ < $CH_3CO_2^-$ of decreasing hydrophobicity (Figure 5) is in line with a hydrophobic binding site afforded by **1c** in polar solvents.

In agreement with this assumption is also the observation of the anion binding ability of the structurally related aliphatic model molecule **3** (Figure 1). Replacing one aryl ring in **1** by an aliphatic CH_3 leads to **3**, whose anion (AcO^-, F^-, and $H_2PO_4^-$) binding constants drop dramatically from 10^6 M^{-1} orders of magnitude in pure MeCN to extremely low values in

TABLE 3: Solvent-polarity Dependent Chemical Shift Differences ($\Delta\delta$) between 1c, 2a and 3 in CD_3CN and D_2O-CD_3CN

	$\Delta\delta$, ppm								
	o-H			*m*-H			*p*-H		
	CD_3CN	20%D_2O−CD_3CN	40%D_2O−CD_3CN	CD_3CN	20%D_2O−CD_3CN	40%D_2O−CD_3CN	CD_3CN	20%D_2O−CD_3CN	40%D_2O−CD_3CN
1c	0.005[a]	−0.005[a]	−0.025[a]	0.009[a]	0.001[a]	−0.001[a]	0.012[a]	0.004[a]	−0.012[a]
		−0.01[b]	−0.03[c]		−0.008[b]	−0.01[c]		−0.008[b]	−0.024[c]
2a	0.014[d]	−0.005[d]	−0.017[d]	0.002[d]	−0.004[d]	−0.004[d]	0.002[d]	−0.002[d]	−0.019[d]
		−0.019[b]	−0.031[c]		−0.006[b]	−0.006[c]		−0.004[b]	−0.021[c]

[a] $\Delta\delta$ between those of **1c** and **3**. [b] Change of $\Delta\delta$ in 20% D_2O-CD_3CN to that in CD_3CN. [c] Change of $\Delta\delta$ in 40% D_2O-CD_3CN to that in CD_3CN. [d] $\Delta\delta$ between those of **2a** and **3**.

TABLE 4: Anion Binding Constants of 1c in pH 5.5 $H_2O-MeCN$ of Varied f_{H2O}[a]

		K_a, M^{-1}		
v/v, %	f_{H_2O}	$CH_3CO_2^-$	$CH_3CH_2CO_2^-$	$C_6H_5CO_2^-$
10	0.24	$(1.66 \pm 0.14) \times 10^5$	$(1.21 \pm 0.54) \times 10^5$	$(8.76 \pm 0.73) \times 10^4$
50	0.74	$(1.30 \pm 0.06) \times 10^4$	$(1.66 \pm 0.05) \times 10^4$	$(1.26 \pm 0.08) \times 10^4$
80	0.92	$(2.80 \pm 0.18) \times 10^3$	$(6.75 \pm 0.59) \times 10^3$	$(5.14 \pm 0.55) \times 10^3$

[a] Anion existed in ammonium acetate, sodium propionate, and sodium benzoate.

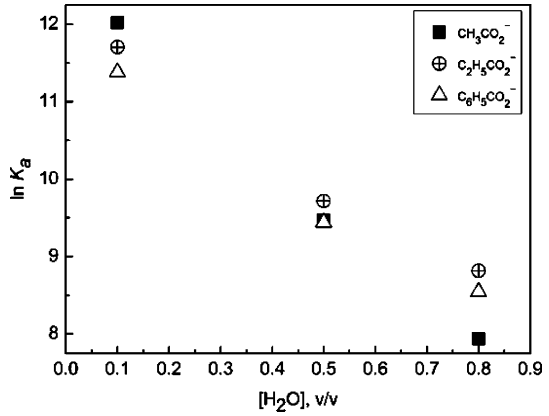

Figure 5. Plots of binding constants of **1c** with $CH_3CO_2^-$, $CH_3CH_2CO_2^-$, and $C_6H_5CO_2^-$ in $H_2O-MeCN$.

$H_2O-MeCN$ so that no noticeable anion binding could be detected at a water volume content of only 10%.

4.2. Effect of −NH Acidity. *4.2.1. Thioureido −NH Acidity.* It should be noted that the anion binding ability of the hydrogen-bonding site itself might still be a factor that influences anion binding in water. The AcO^- binding constants of **1a**−**e** in $H_2O-MeCN$ were found to be correlated linearly to the Hammett constant of substituent (X) at the *N*′-phenyl ring by equations $\ln K_a = 9.50 + 0.62\ \sigma_X$ ($n = 5$, $R = 0.9915$) and $\ln K_a = 8.60 + 0.86\ \sigma_X$ ($n = 5$, $R = 0.9922$) at water volume fractions of 70% and 90%, respectively. This means that the anion binding constant of **1** increases with increasing electron withdrawing ability of X. It was therefore made clear that the acidity of the thioureido −NH protons tunable by the substituent X was still a factor influencing anion binding of **1a**−**e** in aqueous solutions, as is the case with **2** in organic solvents such as MeCN, despite being to a much lower extent.[15]

4.2.2. Amido −NH Acidity. On the basis of the available linear correlation between chemical shifts of the amido −NH protons in *N*-(substituted-benzamido)-*N*′-phenylthioureas (**2**) versus the Hammett substituent constant,[14] it was found that the pyridine ring in **1c** corresponded to a phenyl ring bearing a highly electron-withdrawing *para*-substituent of Hammett constant 0.75. In order to probe whether the amido −NH acidity influences anion binding in aqueous solutions, the binding properties of model molecules **2a**−**e** (Figure 1) were examined, since a hydrophobic cleft could similarly be expected to form between the two phenyl rings in **2a**−**e**. We found that when the substituent Y at the *N*-benzamido phenyl ring of **2** is H (**2a**) or *m*-Cl (**2b**), no anion binding can be detected when the water content was 20% (v/v) in $H_2O-MeCN$ (Table 5). Only when Y is electron-withdrawing strong enough in the case of **2c**−**2e** do they show anion binding capacity in $H_2O-MeCN$ (Table 5). This implies that the amido −NH should be acidic enough to afford the capability of anion binding in aqueous solutions. As the pyridine moiety in **1** can be acidified into pyridinium, which would consequently increase the acidity of the amido −NH proton, anion binding of **1c** in 50% $H_2O-MeCN$ was tested under varied pH. With AcO^- and F^- of substantially differed basicity, anion binding was found completely suppressed when the solution pH was 3.5 or lower (Figures 6 and 7; similar profiles noted in pure water, data not shown). A pK_a value of 3.67 was reported for the pyridinium in isonicotinamide in water,[23] and a pK_a of 3.5 was determined for **1c** in 50% $H_2O-MeCN$ from an absorption spectral titrations. Obviously, when the pyridine N is protonated, the positive charge in the pyridinium prevents its approach to the *N*′-phenyl terminal as the hydrophobic interaction is substantially weakened. In this case, the less polar microenvironment does not exist any more

TABLE 5: Receptor−AcO^- Binding Constants in pH 5.5 $H_2O-MeCN$ of Varied Water Volume Fraction

	K_a (AcO^-), M^{-1}		
R	0.20[a]	0.50[a]	0.80[a]
1c	$(5.11 \pm 0.16) \times 10^4$	$(1.30 \pm 0.06) \times 10^4$	$(2.80 \pm 0.18) \times 10^3$
2a	[b]	[b]	[b]
2b	[b]	[b]	[b]
2c	$(4.86 \pm 0.33) \times 10^4$	$(4.40 \pm 0.36) \times 10^3$	$(5.02 \pm 0.77) \times 10^3$
2d	$(4.35 \pm 0.26) \times 10^4$	$(1.43 \pm 0.05) \times 10^4$	$(4.22 \pm 0.17) \times 10^3$
2e	$(1.29 \pm 0.21) \times 10^5$	$(7.11 \pm 0.21) \times 10^3$	$(1.35 \pm 0.38) \times 10^3$

[a] The water volume fraction in $H_2O-MeCN$ mixtures. [b] Not available because of the minor spectral change.

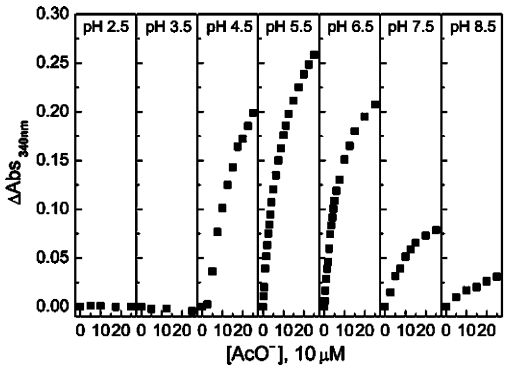

Figure 6. Plots of absorbance of the new band at 340 nm of **1c** against AcO^- concentration in a 50% $H_2O-MeCN$ mixture of varied pH. AcO^- existed in NH_4OAc. [**1c**] = 20 μM.

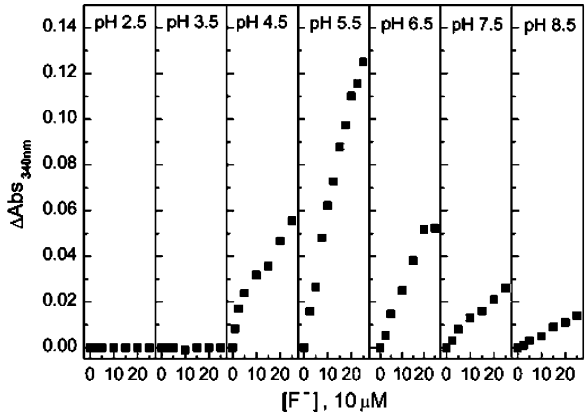

Figure 7. Plots of absorbance of the new band at 340 nm of **1c** against F^- concentration in a 50% H_2O−MeCN mixture of varied pH. F^- existed in NaF. [**1c**] = 20 μM.

in **1c**, and anion binding in aqueous solutions is consequently suppressed despite a more acidic amido −NH.

The requirement that the amido −NH proton in *N*-benzamidothioureas such as **1** and **2** be acidic enough to allow for an efficient anion binding in H_2O−MeCN actually agrees with our previous suggestion that upon anion binding to the thiourea moiety in **2**, a hydrogen-bonding network is formed involving the amido −NH proton and thiourea S atom.[15] As an S atom is not a good hydrogen bonding acceptor, the amido −NH proton shall be more acidic to facilitate the hydrogen bonding interaction. This role of the acidic amido −NH proton in maintaining a hydrogen-bonding network was supported by the fact that the absorption and emission of lactam **4** (Figure 1),[17] a derivative of **1** and **2** but without amido the −NH proton, showed essentially no response toward anions such as AcO^- and F^- in MeCN.

5. Conclusions

We found that simple synthetic neutral receptors, *N*-(isonicotinamido)thioureas (**1a**−**e**) bearing a pyridine moiety, showed substantial anion binding capacity in H_2O−MeCN binary aqueous solutions up to pure water. The pyridine ring here was shown to correspond to a phenyl ring bearing an electron-withdrawing *para*-substituent of Hammett constant 0.75. Accordingly, we found that *N*-(substituted-benzamido)-*N*′-phenylthioureas (**2c**−**e**) bearing a substantially electron-withdrawing substituent at the *N*-benzamido phenyl ring were also able to bind anions in aqueous solutions. Although this seems to point to the critical role of the acidity of the amido −NH proton, we show that it is instead the less polar microenvironment afforded by the approach of two aromatic rings in **1** and **2c**−**e** facilitated by the hydrophobic interactions that promotes the anion binding in aqueous solutions. As the *N*-benzamido phenyl ring in **2** needs to bear an electron-withdrawing substituent in order to enable anion binding in aqueous solutions, it appears that electrostatic interaction between the two phenyl rings may also contribute to their approach.[24] We therefore provided a new clue for designing *N*-amidothiourea-based simple neutral receptors for anion binding in aqueous solutions by taking into account the receptor molecular conformation. It is of relevance to point out that Gunnlaugsson et al.[25] recently reported that an *N*-naphthylaminothiourea showed efficient anion binding in 1:1 H_2O−EtOH at pH 7.4, which deserves further efforts for clarifying detailed mechanisms. As thioureas have recently been shown to be potential organocatalysts in asymmetric syntheses, it is hoped that this clue would also be of significance for developing smart thiourea-based organocatalysts.[26,27]

Acknowledgment. We thank the support of National Science Foundation of China through Grant Nos. 20675069 and 20425518. NFFTBS is gratefully acknowledged for supporting undergraduate training (H.S. and H.-H. H.) via Grant No. J0630429.

References and Notes

(1) Sessler, J. L.; Gale, P. A.; Cho, W.-S. *Anion Receptor Chemistry*; RSC Publishing: Cambridge, U.K., 2006.

(2) For recent comprehensive reviews, see Gale, P. A., Ed. Special issue on anion sensing and recognition. *Coord. Chem. Rev.* **2006**, *250*, 2917−3244.

(3) For a most recent review, see Gale, P. A.; García-Garrido, S. E.; Garric, J. *Chem. Soc. Rev.* **2008**, *37*, 151−190.

(4) Amendola, V.; Esteban-Gómez, D.; Fabbrizzi, L.; Licchelli, M. *Acc. Chem. Res.* **2006**, *39*, 343−353.

(5) Gale, P. A. *Acc. Chem. Res.* **2006**, *39*, 465−475.

(6) Evans, L. S.; Gale, P. A.; Light, M. E.; Quesada, R. *Chem. Commun.* **2006**, 965−967.

(7) Blanco, J. L. J.; Benito, J. M.; Mellet, C. O.; Fernández, J. M. G. *Org. Lett.* **1999**, *1*, 1217−1220.

(8) Blanco, J. L. J.; Bootello, P.; Benito, J. M.; Mellet, C. O.; Fernández, J. M. G. *J. Org. Chem.* **2006**, *71*, 5136−5143.

(9) Rodráguez-Lucena, D.; Benito, J. M.; Mellet, C. O.; Fernández, J. M. G. *Chem. Comm.* **2007**, 831−833.

(10) Kubik, S.; Reyheller, C.; Stüwe, S. *J. Inclusion Phenom. Macrocyclic Chem.* **2005**, *52*, 137−187 and references cited therein.

(11) Chakrabarti, P.; Bhattacharyya, R. *Prog. Biophys. Mol. Biol.* **2007**, *95*, 83−137.

(12) Rodriguez-Docampo, Z.; Pascu, S. I.; Kubik, S.; Otto, S. *J. Am. Chem. Soc.* **2006**, *128*, 11206−11210.

(13) Wu, F.-Y.; Li, Z.; Wen, Z.-C.; Zhou, N.; Zhao, Y.-F.; Jiang, Y.-B. *Org. Lett.* **2002**, *4*, 3203−3205.

(14) Nie, L.; Li, Z.; Han, J.; Zhang, X.; Yang, R.; Liu, W.-X.; Wu, F.-Y.; Xie, J.-W.; Zhao, Y.-F.; Jiang, Y.-B. *J. Org. Chem.* **2004**, *69*, 6449−6454.

(15) Wu, F.-Y.; Li, Z.; Guo, L.; Wang, X.; Lin, M.-H.; Zhao, Y.-F.; Jiang, Y.-B. *Org. Biomol. Chem.* **2006**, *4*, 624−630.

(16) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanavakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Andres, J. L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.9; Gaussian, Inc.: Pittsburgh, PA, 1998.

(17) Yang, Y. K.; Yoon, K. J.; Tae, J. *J. Am. Chem. Soc*. **2005**, *127*, 16760−16761.

(18) Boiocchi, M.; Boca, L. D.; Gómez, D. E.; Fabbrizzi, L.; Licchelli, M.; Monzani, E. *J. Am. Chem. Soc*. **2004**, *126*, 16507−16514.

(19) Connors, K. A. *Binding Constants. The Measurement of Molecular Complex Stability*; John Wiley & Sons: New York, 1987.

(20) Jorgensen, W. L. *J. Am. Chem. Soc.* **1989**, *111*, 3770−3771.

(21) Hefter, G.; Marcus, Y.; Waghorne, W. E. *Chem. Rev.* **2002**, *102*, 2773−2836.

(22) Susumu, K.; Kunimoto, K.; Segawa, H.; Shimidzu, T. *J. Phys. Chem.* **1995**, *99*, 29−34.

(23) Ghosh, S. K.; Balasubramanian, P. N.; Pillai, G. C.; Gould, E. S. *Inorg. Chem.* **1991**, *30*, 487−491.

(24) Gung, B. W.; Wekesa, F.; Barnes, C. L. *J. Org. Chem.* **2008**, *73*, 1803−1808.

(25) Gunnlaugsson, T.; Kruger, P. E.; Jensen, P.; Tierney, J.; Ali, H. D. P.; Hussey, G. M. *J. Org. Chem.* **2005**, *70*, 10875−10878.

(26) For comprehensive recent reviews on organocatalysis, see the special issue edited by List, B. *Chem. Rev.* **2007**, *107*, 5413−5883.

(27) For the most recent examples of thiourea-based organocatalysts, see (a) Zuend, S. J.; Jacobsen, E. N. *J. Am. Chem. Soc*. **2007**, *129*, 15872−15883. (b) Chen, W.; Du, W.; Duan, Y.-Z.; Wu, Y.; Yang, S.-Y.; Chen, Y.-C. *Angew. Chem., Int. Ed.* **2007**, *46*, 7667−7670. (c) Zhang, Z.; Schreiner, P. R. *Synlett*. **2007**, 1455−1457. (d) Connon, S. J. *Chem.—Eur. J.* **2006**, *12*, 5418−5427.