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Fluorescent Anion Sensor Derived from Cholic Acid: The Use of Flexible Side Chain

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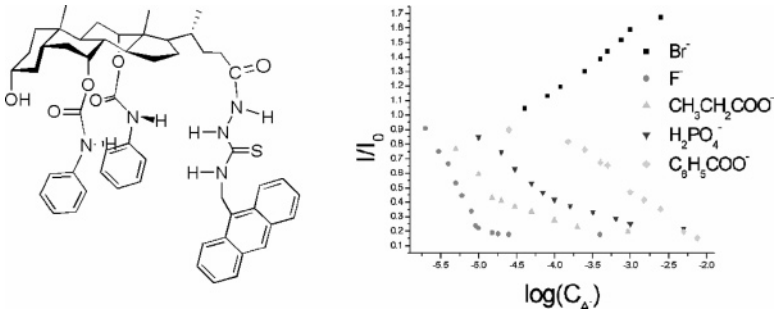

The fluorescent photoinduced electron transfer (PET) chemosensors **1**−**3** were synthesized from cholic acid. **1** and **2** containing amidothiourea groups as anion receptive sites demonstrated much higher affinity toward anions than **3** containing traditional thiourea H-bond donating group. Comparative studies on their binding affinity toward carboxylates, dihydrogen phosphate, and halides revealed that the amidothiourea moiety on the C17 side chain could work cooperatively with H-bond donating groups on C7 and C12 to bind spherical halogen anions. An unexpected specific fluorescence enhancement of **1** by coordinating bromide ion was observed.

Introduction

Anion sensing has attracted increasing attention from various disciplines recently.[1] Molecular recognition of anionic species plays a pivotal role in a large number of biological processes. However, practical detection of anions has continued to be a more challenging issue than the detection of cations, which has been fully developed for almost 40 years.[2] The variety of geometric shapes of anions and synthetic difficulty in building selective anionic receptive sites make the design and synthesis of anion sensor intellectually more demanding and practically less predictable.[1e] Recently, neutral anion receptors embracing hydrogen donor groups have been extensively studied because of the absence of counteranion interferences and the nature of H-bond providing a directional interaction.[3] Notwithstanding these achievements, anion receptors with a built-in signal transduction unit capable of producing easily detectable signal after selective binding are less common.[4] Furthermore, to excel their performance, anion sensors still need improvement in their selectivity and sensitivity. In this article, we report

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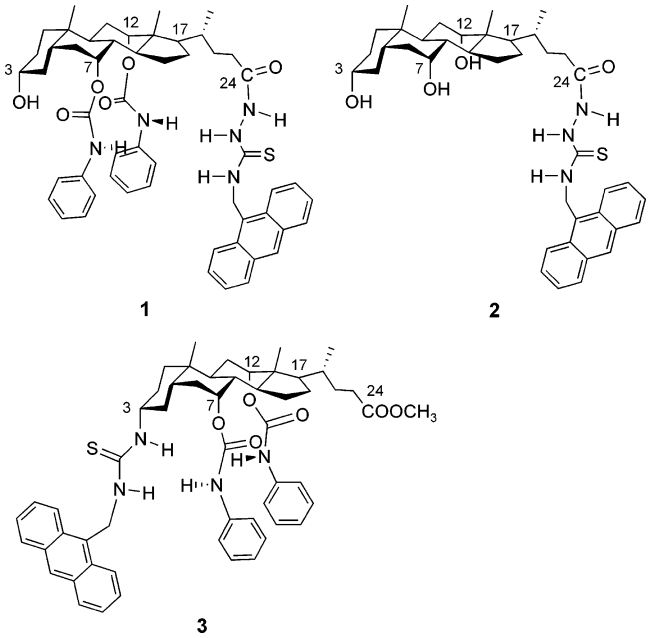

FIGURE 1. Synthetic anion sensors **1**, **2**, and **3**.

the facile synthesis of fluorescent anion sensors **1**−**3** (Figure 1) developed from cholic acid, which have strong fluorescent response, high sensitivity, and specific selectivity toward guest anions.

Davis made a seminal contribution in developing cholate-based receptors, which promise to provide a variety of excellent supramolecular properties.[5] To obtain high affinity and selectivity, the three axially oriented functional groups on cholic acid (C3, C7, and C12) were ingeniously assembled in such a way as to confer a cooperative binding interaction to the guest anion. To the best of our knowledge, in the literature, no attention has been focused on mobilizing the flexible C17 side chain of cholic acid as a chelating group. Examination of a molecular model revealed that in cooperation with the functionalized C7 and C12 pending ligating groups, the template effect provided by a potential anion via three point interactions may trigger the creation of an anionic receptive site with the crucial participation of the C17 functionalized flexible side chain. Attempting to make use of the flexible functional receptive site on C17 and to investigate its potential cooperation effect with C7 and C12 in designing sensor **1**, we built an effective anion receptive amidothiourea group on the C24 carboxyl group of cholic acid.[6] To demonstrate the focused chelating ability of the flexible C17 side chain, the C3 hydroxyl group was deliberately kept intact while the more proximate C7 and C12 hydroxyl groups were functionalized as carbamates serving as H-bond donors. Furthermore, to introduce the signal display element onto the sensor scaffold, we placed anthracene moiety as a typical emissive photoinduced electron transfer (PET) functional unit on the molecule. Therefore, the "switch on−off" property could be detected when the receptive binding site interacts selectively with the guest molecules or ions.[7]

Results and Discussion

On the basis of the aforementioned design strategy, **1** was obtained in moderate yield via a five-step synthetic sequence. Its analogue **2**, lacking the C7 and C12 carbamate groups, was assembled through a similar procedure. Additionally, a typical C-3, 7, 12 tripodal thiourea sensor **3** was also prepared for comparative study. The synthetic routes are shown in Scheme 1.

Starting from commercially available starting material, **4** was treated with methyl acetate to protect the 3α-hydroxyl group and carboxylic group of cholic acid.[8] In the presence of TMSCl, the 7α- and 12α-hydroxyl groups of **4** reacted readily with phenylisocyanate to give **5** in 82% yield. Intending to prepare the carboxyl hydrazide, we regenerated the 3α-hydroxyl group under basic conditions, and the resultant hydroxyacid was then esterified with methyl bromoacetate, affording **6**. **6** was subsequently treated with excess hydrazine hydrate in methanol to give **7** in 87% yield. The appendage of the fluorescent moiety onto **7** was accomplished by its reaction with 9-isothiocyanomethyl-anthracene to give the adduct **1** in 54% yield. By a similar procedure, compound **2** was prepared in 32% overall yield as an analogue of **1** with hydroxyl groups on C-7 and C-12 being intact. On the other hand, our synthetic route to **3** began with the preparation of 3α-azido-methyl cholate **10** according to the reported protocol developed by Davis's group.[5e] After conversion of the 7α- and 12α-hydroxyl groups of **10** to biscarbamate by reacting with excess phenylisocyanate, the azido group of resultant product **11** was reduced by zinc powder to afford 3α-amino derivative. Treatment of **12** by 9-isothiocyanomethylanthracene in dichloromethane at room temperature gave the corresponding thiourea **3** in 25% yield. All the new compounds and key intermediates were fully characterized by ^{1}H NMR, ^{13}C NMR, IR, as well as HRMS.

Since these sensors are designed as H-bond based receptors, acetonitrile lacking the ability to form H-bond with sensors was chosen as the solvent in the recognition investigation. In fluorescence study, **1**, **2**, and **3** were excited at 366 nm and gave out a characteristic emission maximum at ca. 413 nm. All the fluorescent emissions of **1**, **2**, and **3** can be quenched by different anions in the form of tetraalkylammonium salts without significant

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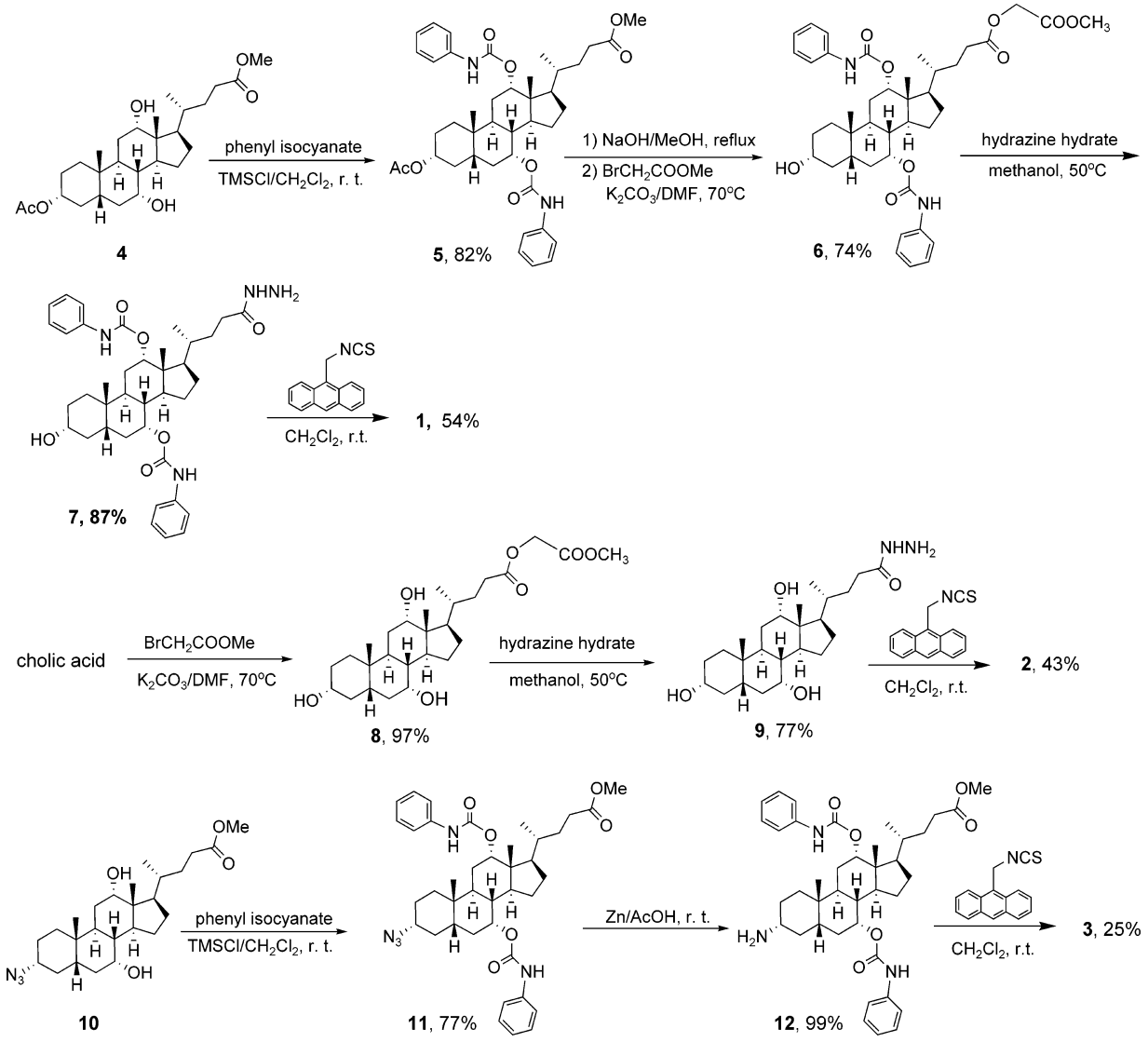
SCHEME 1. Synthetic Route for 1, 2, and 3



change in their UV−visible absorption spectra (Figure 2a). The observed fluorescent quench could be attributed to the typical guest-induced PET process.[7a] Upon anion coordination with the sensor, the reduction potential of binding moieties (i.e., thiourea or amidothiourea group) would increase, leading to the enhancement of a PET effect, which is responsible for the emission quenching of compounds **1**, **2**, and **3**.[1a,7] Upon adding 5% water to the work solution, all the fluorescent responses are diminished because of water's propensity to act as a strong H-bond donating competitor.

Quantitative investigations of the binding behaviors of **1**, **2**, and **3** were performed with different anions in acetonitrile by means of titration fluorimetry. On the basis of the change of fluorescent intensity in stepwise adding guest anions, the complex association constants (K_a) were calculated using nonlinear least-squares curve fitting[9] (Table 1). Excellent fitting ($R > 0.99$) of the titration data points shown in Figure 3 show that **1**, **2**, and **3** invariantly form 1:1 complexes with carboxylates, dihydrogen phosphate, or bromide.[6a,9,10] To bind different carboxylates, the affinity of **1**, **2**, or **3** is correlated well with their basicity. The sequence of the K_a values (propionate > acetate > benzoate > lactate) reflects the decreasing intrinsic basicity of the respective carboxylates. Chloride was claimed to be a compatible anion that can be fitted in the cholate cavity; however, in this study, except for a slight fluorescence enhancement of **1**, we could not observe significant change in emission intensity of the sensors upon adding excess amount of chloride. Similar to the response of chloride, bromide can only increase the fluorescent intensity of **1** without changing emission spectra of **2** and **3**. To make a complete study on halide ions, iodide titrations were also conducted. It was found that all the sensors exhibited no fluorescent response to a moderate concentration of iodide ($c_I < 10^{-3}$ mol/L). However, when c_I was higher than 10^{-3} mol/L, the heavy atom effect of iodine became apparent and the fluorescent intensities of **1**, **2**, and **3** were all quenched by iodide via intersystem crossing.[11]

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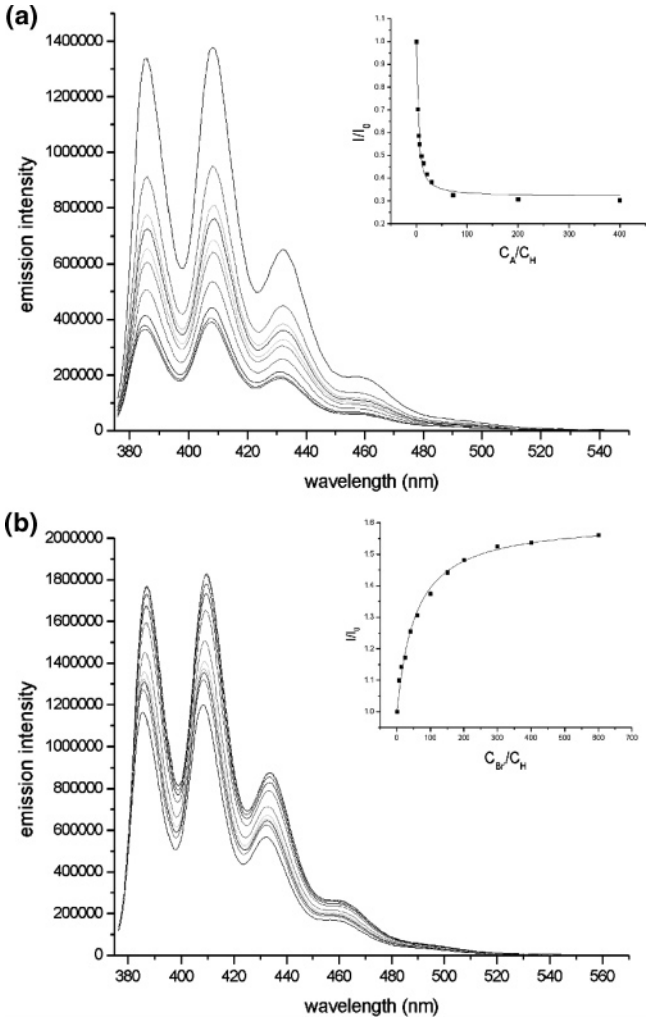

FIGURE 2. Fluorescence response of **1** (5 μM) by adding (a) TBA acetate and (b) TBA bromide in acetonitrile. λ_{ex} = 366 nm. Inset is the titration data point and the nonlinear least-squares fitting curve.

To each of the tested anions, compounds **1** and **2** showed much larger binding constants than that of compound **3**, indicating that amidothiourea group has higher H-bond donating capacity (stronger acidity) than thiourea does. Reminiscent of the findings of others, the intramolecular hydrogen bond present in **1** could enhance the planarity of the amidothiourea group leading to a stronger binding.[6a,b] Moreover, as one of the strongest H-bond acceptors, F^- exerts essentially a different interaction with amidothiourea and thiourea H-bond donors. The stoichiometrical ratio of sensor and F^- was determined to be 2:1 by Job plot experiment when **1** or **2** was titrated by fluoride (Figure 3) while a 1:1 ratio was found in titration of **3** and F^-. Such different binding behaviors could be originated from the variation of the N−H bond acidity of thiourea and amidothiourea. The more acidic amidothiourea subunit tends to undergo an advanced stage of proton transfer after typical H-bond addition.[12] Such stepwise equilibria, illustrated by the equation below where LH = **1** or **2**, consume two fluoride ions for each amidothiourea−methylene anthracene moiety, giving rise to a substantial PET quenching.

$$[LH{\cdot}F]^- + F^- \rightleftharpoons L^- + [HF_2]^-$$

Comparison between the binding properties of **1** and **2** toward other different anions gave us a clear understanding on the nature of the binding. For trigonal planar carboxylates and tetrahedral $H_2PO_4^-$, their respective binding constants with **1** and **2** are on the same magnitude, indicating that the H-bond interaction only occurs on amidothiourea group, while for spherical anions such as fluoride and bromide, the presence of the 7α- and 12α-carbamate groups in **1** is apparently essential. Even the second-order association constants of **1**·F^- and **2**·F^- are too large to calculate; a much acuter response of **1** toward fluoride in the titration curves indicates that **1** boasts much higher affinity toward fluoride than **2** does (Figure 3a). Moreover, fluorescent intensity of **1** can be drastically enhanced by bromide while such a phenomenon turns to be marginalized on **2** under the same experimental condition. It is clear that cooperation effect between the C7, C12 carbamates and the amidothiourea on C17 side chain led to the large difference between **1** and **2** in halide binding ability. Although fluoride demonstrated a 2:1 coordination behavior, there is no doubt that the presence of a multi H-bond donor in **1** would stabilize the formation of the initial $[LH{\cdot}F]^-$ complex and thus facilitate the advanced deprotonation. For **1**, the free rotating C17 side chain donating H-bond could be assembled with the C7, C12 carbamates under anionic template effect to achieve more stable three point coordination[13] without costing much energy. Such assembly could only happen on spherical halide anions on which negative charge is distributed symmetrically on the surface, while for carboxylates and dihydrogen phosphate, the CO_2^- and PO_2^- subunits serving as directional H-bond acceptor can hardly interact with additional H-bond donor after

TABLE 1. Association Constants (K_a) of Coordination between 1, 2, and 3 with Different Anions (M^{-1})

anions	1	2	3
fluoride	*a*	*a*	$(2.07 \pm 0.38) \times 10^4$
chloride	8% enhancement[b]	no response	negligible quenching[b]
bromide	$(3.67 \pm 0.38) \times 10^3$	no response	negligible quenching[b]
iodide	no response when $c_I < 10^{-3}$ mol/L, heavy atom quenching when $c_I > 10^{-3}$ mol/L		
dihydrogen phosphate	$(3.11 \pm 0.17) \times 10^4$	$(2.72 \pm 0.17) \times 10^4$	$(1.82 \pm 0.32) \times 10^3$
propionate	$(1.60 \pm 0.15) \times 10^5$	$(5.59 \pm 0.47) \times 10^4$	$(5.93 \pm 1.24) \times 10^4$
acetate	$(7.69 \pm 0.97) \times 10^4$	$(4.13 \pm 0.30) \times 10^4$	$(1.18 \pm 0.12) \times 10^4$
benzoate	$(7.10 \pm 0.67) \times 10^3$	$(6.12 \pm 0.58) \times 10^3$	475 ± 46
lactate	381 ± 46	626 ± 20	no response

[a] The second-order association constant was too large to be calculated. [b] The associate constant could not be calculated precisely because the signal change was too low to provide reliable data with tolerable error.

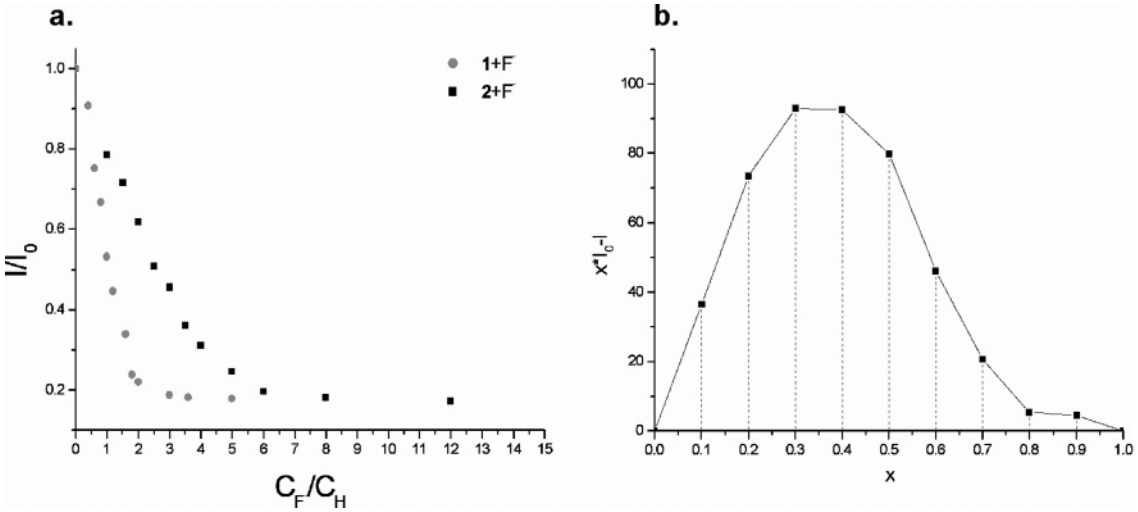

FIGURE 3. (a) Fluorescence quench of **1** (5 μM) and **2** (5 μM) by titrating F^-. (b) The Job plot of **1** and F^- indicating 2:1 coordination (x is the mole fraction of **1**).

coordination with amidothiourea. This presumption was further substantiated by ^{1}H NMR study of **1** in the presence and absence of different anions in CD_3CN. The N−H hydrogen bond signals of the free host **1** were masked by the aromatic protons in 6.8−8.5 ppm. After addition of 10 equiv of tetrabutylammonium acetate, only two new peaks were found in the downfield (i.e., 11.3−11.7 ppm), which were ascribed to anion-coordinated amidothiourea N−H protons. In contrast, addition of 10 equiv of tetrabutylammonium chloride or bromide led to the appearance of four new peaks in 8.7−10.1 ppm. These new signals should be ascribed to the combination of N−H signals of amidothiourea and carbamates that underwent a cooperative binding of chloride or bromide.[14]

It is interesting to find that Br^-, which is rarely reported as a PET quencher, could enhance the emission intensity of **1** for as much as 60% in acetonitrile (Figure 2b). But such phenomenon could not be observed on **3** or on the analogue compound **2** under the same experimental conditions. Moreover, the observed bromide-induced fluorescent enhancement of **1** became much weaker when the experiment was undertaken in other solvent such as CH_2Cl_2 and toluene (Figure 4). Conceivably, amidothiourea, being a powerful receptive site for anion, could coordinate the polar CH_3CN solvent molecules triggering considerable PET fluorescent quenching on anthracene moiety.[15] We believe that compound **1** does coordinate with Cl^- and Br^- via cooperative multi H-bond donors. Upon addition of chloride or bromide, due to their stronger binding affinity to **1** via multi H-bonding interaction, they could readily break the CH_3CN solvation to form stable complexes with the host. It is noteworthy that chloride and bromide anions are much less negative

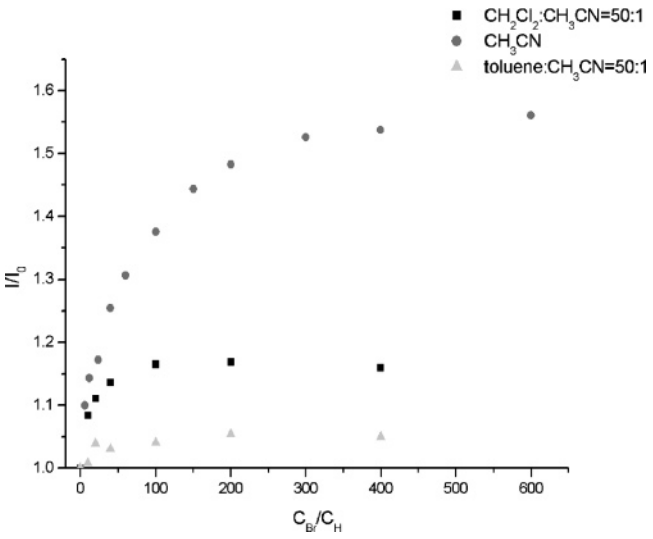

FIGURE 4. Titration curves of **1** (5 μM) and Br^- in different solvents.

in charge than fluoride and oxygen anions; as a result, they can hardly change the reduction potential of amidothiourea even upon binding. The effect of chloride on inducing quenching on **1** via PET may be comparable to that of CH_3CN, and thus only 8% emission enhancement of **1** was detected upon binding with chloride. When CH_3CN is replaced by the less negative bromide ion, the preexisted PET process due to the polar solvent will be erased. As a result, an enhancement of fluorescence intensity was observed.

Conclusion

In summary, we have developed a novel efficient anion-detecting system via an effective synthetic route to construct H-bond donor group basing on cholic acid scaffold. Extensive study of these synthetic sensors' fluorescence response to different anions demonstrated their excellent selectivity and affinity toward negatively charged substrates. Rationally designed experiments manifested the mechanism for fluorescent change of the

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chemosensor showing supramolecular cooperation among the flexible C17 side chain, the C7, and the C12 axially oriented arms of the molecule. The preorganized C3 functional site could be utilized to construct an additional binding site. The design of ion pair chemosensors along these tactics has been underway.

Experimental Section

Methyl 3α-Acetoxy-7α,12α-bis(phenylaminocarbonyloxy) Cholate (5). To a solution of **4** (260 mg, 0.56 mmol) being stirred in freshly distilled dry dichloromethane, a drop of trimethylsilane chloride and redistilled phenylisocyanate (123 μL, 1.1 mmol) was added at room temperature. After being stirred for 24 h, another 123 μL of phenylisocyanate was injected to the solution. The solid was filtered out after **4** had disappeared on TLC. Removing of solvent was followed by flash column chromatography (SiO_2) eluted by petroleum ether and ethyl acetate (3:1) that gave the crude product. The product was taken up by small amount of dichloromethane, and the insoluble impurity was filtered out. Compound **5** was obtained after removing of dichloromethane (322 mg, 82%): mp 115−118 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.77 (s, 3H), 0.89 (d, 3H, J = 6.2 Hz), 0.95 (s, 3H), 1.99 (s, 3H), 3.62 (s, 3H), 4.59 (m, 1H), 4.95 (s, 1H), 5.12 (s, 1H), 6.67 (br, 1H), 6.75 (br, 1H), 7.06−7.13 (m, 2H), 7.31−7.48 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.3, 17.5, 21.5, 22.5, 22.9, 25.8, 26.8, 27.1, 29.0, 30.7, 30.9, 31.5, 34.4, 34.6, 34.7, 37.9, 40.8, 43.6, 45.3, 47.4, 51.5, 71.9, 74.1, 118.6, 123.5, 129.0, 138.0, 152.9, 153.1, 170.3, 174.6. FAB MS (*m/z*): $[M]^+$ = 702.2; $[M + H]^+$ 703.2. HRMS: calcd for $C_{41}H_{54}N_2O_8Na$, 725.3777; found, 725.3775.

Methoxycarbonylmethyl 7α,12α-Bis(phenylaminocarbonyloxy) Cholate (6). 5 (250 mg, 0.35 mmol) was added into a reflux solution of sodium hydroxide (99 mg, 1.8 mmol) in 20 mL of methanol. After being stirred for 1 h, the mixture was cooled to room temperature and stirred overnight. The alkali solution was then neutralized by saturated ammonium chloride solution. Removal of methanol under reduced pressure was followed by extraction of aqueous solution by ethyl acetate. The organic layer was dried, and the solvent was removed to give deprotected bis-carbamate. The crude product was dissolved in dry DMF (15 mL) in the presence of potassium carbonate (97 mg, 0.7 mmol). Methyl bromoacetate (33 μL, 0.35 mmol) was then added by microsyringe. After reacting at 70 °C for 15 h, the mixture was poured into 30 mL of saturated ammonium chloride solution and then extracted by three portions of ethyl acetate. Product **6** was obtained as white foam after drying and removal of organic solvent (185 mg, 74%). mp 114−122 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.78 (s, 3H), 0.86−0.93 (m, 9H), 3.52 (m, 1H), 3.73 (s, 3H), 4.57 (d, 2H, J = 2.4 Hz), 4.96 (s, 1H), 5.13 (s, 1H), 6.84 (br, 1H), 6.91 (br, 1H), 7.04−7.09 (m, 2H), 7.29−7.48 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.3, 14.1, 17.5, 22.6, 22.9, 26.0, 27.2, 27.7, 28.2, 29.4, 30.5, 31.1, 31.5, 34.6, 34.8, 35.4, 35.8, 37.9, 43.6, 45.4, 47.4, 52.2, 60.4, 66.5, 72.4, 118.6, 123.5, 129.0, 129.1, 138.0, 153.1, 168.4, 173.4. FAB MS (*m/z*): $[M + H]^+$ = 718.3. MALDI TOF HRMS: calcd for $C_{41}H_{54}N_2O_9Na$, 741.3727; found, 741.3703.

7α,12α-Bis(phenylaminocarbonyloxy) Cholic Hydrazide (7). The stirring mixture of **6** (170 mg, 0.24 mmol) and hydrazine (0.3 mL, large excess) monohydrate in methanol (10 mL) was heated to 50 °C for 12 h. Methanol was then removed, and the residue was taken up by ethyl acetate. The organic solution was first washed by saturated ammonium chloride solution (3 × 30 mL) and then dried by sodium sulfate. Pure product **7** was obtained after removal of organic solvent (137 mg, 87%): mp 170−175 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.76 (s, 3H), 0.87−0.92 (m, 9H), 3.51 (m, 1H), 4.94 (s, 1H), 5.12 (s, 1H), 7.04−7.11 (m, 2H), 7.29−7.48 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.3, 14.1, 17.6, 22.6, 22.9, 26.0, 27.2, 27.7, 28.2, 29.4, 30.7, 30.9, 31.2, 31.6, 34.7, 34.9, 35.4, 35.8, 37.9, 43.6, 45.4, 47.4, 51.5, 66.5, 72.5, 118.7, 123.5, 129.1, 138.0, 153.0, 174.6. FAB MS (*m/z*): $[M + H]^+$ = 661.4. HRMS: calcd for $C_{38}H_{52}N_4O_6$, 683.3784; found, 683.3780.

Amidothiourea 1. A mixture of **7** (130 mg, 0.2 mmol) and 9-isothiocyanomethylanthracene (49 mg, 0.2 mmol) was stirred in dry dichloromethane (12 mL) at room temperature for 48 h. Column chromatography (SiO_2) eluted by the solvent of hexane/ethyl acetate/dichloromethane (1:1.5:1) gave the pure product **1** as a pale yellow solid (99 mg, 54%): mp ∼178−182 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.56−0.60 (m, 6H), 0.92 (s, 3H), 3.47 (m, 1H), 4.89 (s, 1H), 4.96 (s, 1H), 5.52 (s, 2H), 6.96−7.45 (m, 14H), 7.94 (d, 2H, J = 7.8 Hz), 8.09 (d, 2H, J = 8.3 Hz), 8.38 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.2, 17.3, 22.4, 22.7, 25.8, 26.7, 28.9, 29.7, 30.2, 31.4, 34.2, 34.4, 34.8, 37.9, 38.3, 40.8, 41.5, 43.3, 45.1, 46.8, 71.7, 76.0, 118.5, 119.1, 123.2, 123.4, 123.6, 125.2, 126.7, 128.129.0, 129.1, 130.3, 131.2, 138.2, 138.4, 153.3, 153.4, 170.8, 180.2. IR (KBr): 3287, 2950, 2868, 1726, 1705, 1600, 1533, 1443, 1313, 1225 cm^{-1}. FAB MS (*m/z*): $[M + H]^+$ = 910.5. MALDI TOF HRMS: calcd for $C_{54}H_{63}N_5O_6SNa$, 932.4397; found, 932.4354.

Methoxycarbonylmethly Cholate (8). To a mixture of cholic acid (613 mg, 1.5 mmol) and potassium carbonate (414 mg, 3 mmol) being stirred in dry DMF (20 mL), methyl bromoacetate (156 μL, 1.7 mmol) was added by microsyringe. After reacting at 60 °C for 15 h, the mixture was poured into 30 mL of saturated ammonium chloride solution and then extracted by ethyl acetate (3 × 30 mL). Product **8** was obtained as a white foam after drying and removal of organic solvent (700 mg, 97%): mp 108−111 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.67 (s, 3H), 0.88 (s, 3H), 0.99 (d, 3H, J = 6.0 Hz), 3.43 (m, 1H), 3.76 (s, 3H), 3.84 (s, 1H), 3.96 (s, 1H), 4.61 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.5, 17.3, 22.5, 23.2, 26.4, 27.4, 28.2, 30.4, 30.7, 34.6, 34.7, 39.5, 39.6, 41.4, 41.7, 46.4, 47.0, 52.2, 60.4, 68.4, 71.9, 73.0, 173.6. FAB MS: *m/z* = 481.7.

Cholic Hydrazide (9). The mixture of **8** (240 mg, 0.5 mmol) and hydrazine monohydrate (0.5 mL, large excess) being stirred in methanol (15 mL) was heated to 50 °C for 12 h. Methanol was then removed, and the residue was purified by flash column chromatography eluted by the mixed solvent of CH_3OH/CH_2Cl_2 (1:3) to get crude product **9** (162 mg, 77%). An analytical sample was obtained by running another column chromatography: mp 172−177 °C. ^{1}H NMR (400 MHz, CD_3OD): δ 0.70 (s, 3H), 0.91 (s, 3H), 1.02 (d, 3H, J = 6.4 Hz), 3.36 (m, 1H), 3.79 (d, J = 2.8 Hz, 1H), 3.94 (s, 1H). ^{13}C NMR (100 MHz, CD_3OD): δ 13.1, 17.7, 23.3, 24.3, 28.0, 28.8, 29.7, 31.3, 32.1, 33.2, 35.9, 36.0, 36.6, 37.0, 40.6, 41.1, 43.1, 43.3, 47.6, 48.1, 69.1, 73.0, 74.1, 176.0. IR (KBr): 3454, 2941, 2863, 1636, 1594, 1076 cm^{-1}. TOF HRMS: calcd for $C_{24}H_{42}N_2O_4Na$, 445.3042; found, 445.3041.

Amidothiourea 2. A mixture of **9** (180 mg, 0.45 mmol) and 9-isothiocyanomethylanthracene (110 mg, 0.45 mmol) was stirred in dry dichloromethane (12 mL) at room temperature for 48 h. Column chromatography (SiO_2) eluted by the solvent of (CH_2Cl_2/methanol, 5:1) gave the pure product **2** as pale yellow solid (130 mg, 43%): mp 185−190 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.46 (s, 3H), 0.58−0.78 (m, 9H), 3.18 (m, 1H), 3.66 (s, 1H), 3.74 (s, 1H), 5.61 (s, 2H), 7.42−7.49 (m, 4H), 7.94 (d, 2H, J = 7.6 Hz), 8.13−8.23 (m, 2H), 8.38 (s, 1H), 9.21 (br, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1, 17.2, 18.0, 22.3, 22.6, 23.0, 26.1, 27.2, 27.6, 30.0, 30.5, 34.5, 34.8, 35.2, 39.3, 41.2, 41.6, 45.3, 46.0, 68.3, 71.6, 73.0, 122.2, 123.7, 124.1, 125.2, 126.7, 127.6, 128.2, 128.9, 130.6, 131.3, 146.5, 181.4. IR (KBr): 3414, 2926, 2863, 1684, 1644, 1525, 1446, 731 cm^{-1}. FAB MS (*m/z*): $[M + H]^+$ = 672.2. MALDI TOF HRMS: calcd for $C_{40}H_{53}N_3O_4SNa$, 694.3654; found, 694.3658.

Methyl 3α-Azido-7α,12α-bis(phenylaminocarbonyloxy) Cholate (11). To a solution of **10** (223 mg 0.5 mmol) being stirred in freshly distilled dry dichloromethane (12 mL), a drop of trimethylsilane chloride and redistilled phenylisocyanate (110 μL, 1 mmol) was added at room temperature. After being stirred for 24 h, another 110 μL of phenylisocyanate was injected to the solution. The solid was filtered out after **10** had disappeared on TLC. Removal of solvent was followed by

column chromatography (SiO_2) that gave the crude product. The product was taken up by a small amount of dichloromethane, and the insoluble impurity was filtered out. Compound **11** was obtained after removal of dichloromethane (263 mg, 77%): mp 82−90 °C. ^{1}H NMR (270 MHz, $CDCl_3$): δ 0.78 (s, 3H), 0.89 (d, 3H, J = 5.9 Hz), 0.95 (s, 3H), 3.18 (m, 1H), 4.95 (s, 1H), 5.13 (s, 1H), 6.66 (br, 1H), 6.73 (br, 1H), 7.07−7.12 (m, 2H), 7.32−7.47 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.2, 17.5, 22.9, 24.6, 25.9, 27.2, 28.5, 29.7, 30.1, 30.7, 30.9, 31.1, 32.4, 34.7, 36.2, 37.9, 43.5, 47.4, 51.5, 58.2, 72.3, 118.6, 123.6, 129.0, 129.1, 137.8, 137.9, 153.0, 174.6. FAB MS: *m/z* = 685.6 (M). MALDI TOF HRMS: calcd for $C_{39}H_{51}N_5O_6Na$, 708.3737; found, 708.3765.

Methyl 3α-Amino-7α,12α-bis (phenylaminocarbonyloxy) Cholate (12). Activated zinc powder (400 mg, 6.2 mmol) was added to a solution of **11** (220 mg, 0.32 mmol) in glacial acetic acid (10 mL). The mixture was stirred vigorously for 24 h. Acetic acid was then completely removed under reduced pressure by adding toluene several times. The residue, acetate ammonium salt of **12**, was dissolved by saturated sodium chloride solution (10 mL). Basification of the solution by excessive triethylamine and extraction of it by ethyl acetate gave amine **12** as the pure product. After being dried by sodium sulfate and removal of organic solvent, compound **12** was obtained as white foam (185 mg, 99%): mp 92−96 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (s, 3H), 0.82−0.92 (m, 6H), 2.67 (m, 1H), 4.93 (s, 1H), 5.13 (s, 1H), 7.02−7.07 (m, 2H), 7.28−7.50 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.3, 14.1, 17.6, 22.6, 22.9, 25.8, 27.2, 28.9, 30.7, 30.9, 31.6, 34.4, 34.7, 35.3, 38.0, 41.0, 43.5, 45.3, 47.4, 51.1, 51.5, 118.7, 123.1, 123.3, 129.0, 138.2, 138.5, 153.1, 174.6. FAB MS (*m/z*): $[M + H]^+$ = 660.3.

Thiourea 3. 12 (180 mg, 0.27 mmol) and 9-isothiocyanomethylanthracene (68 mg, 0.27 mmol) were dissolved in freshly distilled dry dichloromethane (12 mL) and stirred for 20 h. The reaction could not finish completely. The mixed product was purified by column chromatography (SiO_2) eluted by dichloromethane first and then by dichloromethane/ethyl acetate = 8:1 to give **3** as a pale yellow powder (63 mg, 25%): mp 129−133 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.75 (s, 3H), 0.86 (s, 3H), 0.90 (d, 3H, J = 6.4 Hz), 3.07 (m, 1H), 3.31 (s, 3H), 4.95 (s, 1H), 5.29 (s, 1H), 5.55 (dd, 2H, J_1 = 13.8 Hz, J_2 = 5.4 Hz), 6.89−7.59 (m, 17H), 7.92 (d, 2H, J = 11.6 Hz), 8.39 (s, 1H), 9.05 (br, 1H), 9.16 (br, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.5, 17.6, 22.3, 22.8, 26.2, 27.1, 29.1, 30.4, 30.5, 30.9, 31.3, 33.8, 34.6, 35.1, 38.0, 40.9, 42.5, 43.6, 45.1, 47.1, 51.1, 52.8, 70.6, 75.2, 77.2, 118.2, 118.9, 122.8, 123.1, 125.3, 126.1, 127.1, 128.7, 128.8, 129.0, 130.4, 131.2, 138.7, 153.1, 153.8, 174.3. IR (KBr): 3376, 3266, 2947, 1727, 1537, 1218 cm^{-1}. TOF HRMS: calcd for $C_{55}H_{64}N_4O_6NaS$, 931.4444; found, 931.4456.

Fluorescent Response Study. All the host compounds **1**, **2**, and **3** were prepared as stock solutions in acetonitrile for ~$2-5 \times 10^{-4}$ mol/L. Except benzoate and lactate, whose countercation were tetramethylammonium salt, all other anions used in this report were in the form of tetrabutylammonium salt (fluoride used in this report was originally 75 wt % water solution of tetrabutylammonium fluoride). They were prepared to approximately 0.01 mol/L and ~$2-5 \times 10^{-3}$ mol/L of stock solutions in acetonitrile. The work solutions were prepared by adding different volumes of anion solution to a series of test tubes followed by dilution to 5 mL by acetonitrile. Then, the same amount of stock solution of the host compound was added into each of the test tubes. After being shaken for several minutes, the work solutions could be measured immediately.

1:1 Association constants of **1**, **2**, and **3** with anions are calculated by nonlinear least-squares curve fitting using the following equation in Origin 6.0:

$$I/I_0 = 1 + \frac{I_{lim}/I_0 - 1}{2}\left[1 + \frac{C_A}{C_H} + \frac{1}{K_s C_H} - \sqrt{\left(1 + \frac{C_A}{C_H} + \frac{1}{K_s C_H}\right)^2 - 4\frac{C_A}{C_H}}\right]$$

where I_0 is fluorescent intensity of host without anions, I_{lim} is fluorescent intensity reaching a limitation by adding excessive anions, C_A is the concentration of anions added, and C_H is the concentration of host molecule.

By allowing $1/K_aC_H$ and I_{lim}/I_0 to be floating parameters, we can obtain the K_a and I_{lim}/I_0 values by a nonlinear least-squares analysis of I/I_0 versus C_A/C_H.

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Supporting Information Available: Compound characterization spectra and fluorometric titration figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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