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General but Discriminating Fluorescent Chemosensor for Aliphatic Amines

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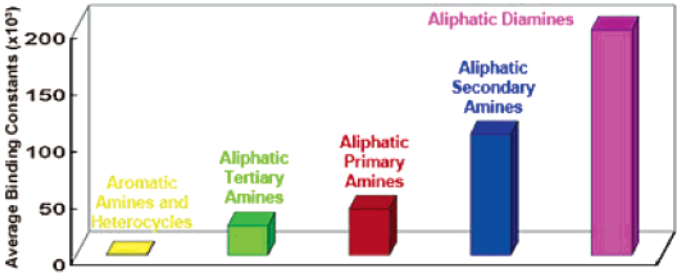

Aliphatic amines are sensitively and discriminatively detected through binding with demethylated naphthol AS-BI (7-bromo-3-hydroxy-2-naphth-*o*-hydroxyanilide, **2**) and fluorescence of the resulting complex. Recognition of the amine by the chemosensor **2** occurs via proton transfer of the naphtholic proton to the amine and is facilitated by the presence of the phenol group. Amine basicity is the primary controller of detection. Poorly basic aromatic and conjugated amines such as pyridine and aniline are not detected. Hydrogen bonding within the complex allows further differentiation of aliphatic amines. Doubly primary, conformationally flexible diamines are the most sensitive to detection, followed by secondary amines.

Introduction

Aliphatic amines are widespread in nature and not often benign. Many biogenic amines such as histamine, cadaverine, and putrescine, so named because of their characteristic odors, are formed by pyrolytic decarboxylation of amino acids. They may constitute toxic components in fish and, hence, can serve as a measure of fish quality.[1−5] Tyramine is a potent bacterial mutagen that induces tumors at multiple sites in rodents.[6,7] Dopamine is a well-known neurotransmitter, and epinephrine (adrenaline) is an adrenal hormone. The entire class of alkaloids comprises toxic amines. Some are used pharmaceutically (morphine, codeine, quinine), others are used addictively (nicotine, cocaine, mescaline), and others are used as deadly poisons [coniine (Socrates's hemlock), strychnine]. Because aliphatic amines are common constituents in the industrial preparation of, inter alia, pharamaceuticals, fertilizers, surfactants, and colorants, they become pollutants in landfills, manufacturing sites, and even the general soil and aqueous environment. In light of the widespread presence of aliphatic amines, the development of effective analytical sensors for these compounds has been an area of considerable interest for many years.

Crown ethers and heterocrown ethers constituted the earliest hosts or chemosensors for amines, which were recognized primarily in their ammonium forms.[8−11] Metalloporphyrin hosts were developed to bind neutral amines by coordination to metal cations.[8−10,12,13] These hosts were able to recognize amines in

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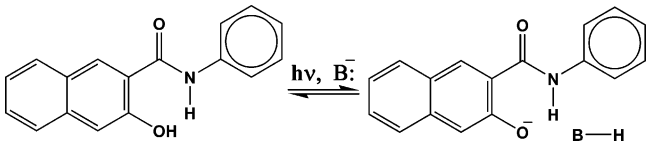
both organic solvents and water.[14,15] Dynamic covalent chemistry added a new dimension to amine recognition, for example, the reaction of neutral, primary amines with carbonyl groups in dyes to form fluorescent imines.[16,17] Zimmerman and co-workers provided an element of selectivity by preparing a molecularly imprinted polymer dye for the detection of diamines.[18,19] A similar approach was used by Greene et al.[20] Functionalized mesoporous silica[21] and conducting organic polymers[23] also have been used to detect amines. Probably the best approach is the use of an array of chemosensors to provide pattern analysis for amines.[23] Such a technology could benefit from the development of new chemosensors with good binding properties.

Despite notable successes, the design and synthesis of amine chemosensors have had limitations. (1) Synthetic hosts may require multistep syntheses, which would be prohibitive in a practical context. For example, Zimmerman's imprinted polymer required a total of 12 synthetic steps.[19] (2) Chemosensors can suffer from low binding constants during recognition. (3) Derivatization in dynamic covalent processes can be a limiting process. Secondary and tertiary amines, for example, cannot form neutral imines and, hence, fail to be detected by this method. It was the objective of the present study to develop a chemosensor for neutral, aliphatic amines (as distinguished from anilines and aromatic amines) without derivatization, without significant chemical synthesis, with large binding constants, and with discriminating ability within this class.

Results

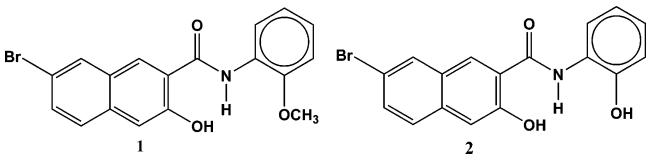
Fluorescence quenching of naphthols by amines has been studied widely, although not in a role as a chemosensor.[24,25] Aliphatic amines effectively quench 1-naphthol, but their excited-state binding constants are relatively low (10−150).[26] We sought a more highly substituted naphthol that would provide fluorescence quenching with stronger binding and, hence, could serve as a useful chemosensor. Our approach was based on the anion sensor developed by Jiang and co-workers.[27] They reported that 3-hydroxy-2-naphthanilide binds to a variety of anions ($B:^-$) via excited-state proton transfer (eq 1).[27,28]



Their study indicated that anions were detected by proton transfer from the naphtholic group rather than from the amidic group in the excited state to form the anion in eq 1, as signaled by an enhanced fluorescent emission. They characterized their observations as constituting "a new signaling mechanism in constructing chemosensors for anions". Our present work was designed to provide a similar advance in the field of amine detection.

Proton transfer, as in eq 1, is enabled by the enhanced acidity of the naphtholic OH upon photoexcitation.[27,29] The ground-state pK_a of 2-naphthol is 9.5 in water, whereas the excited-state pK_a^* is enhanced by nearly seven orders of magnitude, to 2.8.[30] Within the context of our study, excited-state complexes have been observed by proton transfer from 2-naphthol to aliphatic amines.[31] In the ground state, naphthols interact with amines through hydrogen bonding.[32] In the excited state, 1-naphthol has been observed to form 1:1 complexes with aliphatic amines in nonpolar rigid matrixes at low temperature.[31] These authors suggested that the complexes consisted of ion pairs of naphtholate anions and ammonium cations. The interaction is solvent-dependent, however, and may vary from hydrogen-bonded species in cyclohexane[33] to solvent-separated ion pairs in acetonitrile.[34]

We chose a variation of the Jiang et al. molecule[27] for our studies: the commercially available dye naphthol AS-BI (**1**), developed for the cytochemical detection of alkaline phosphatase.[35,36] We also examined the demethylated derivative **2** (7-bromo-3-hydroxy-2-naphth-*o*-hydroxyanilide). Both materials are colorless in the ground state (λ_{max} = 335 nm) but emit fluorescently in the visible region, **1** at 512 nm (weakly) and **2** at 525 nm (more strongly).



We studied the complexation of both **1** and **2** with amines systematically by fluorescence spectroscopy. The addition of aliphatic amines to a solution of **2** has a profound effect on the electronic spectra. The colorless solution of **2** (10 μM) becomes lightly colored (yellow; λ_{max} = 427 nm) on addition of 1,3-diaminopropane (or any number of amines) in acetonitrile, whereas the methoxy dye **1** shows virtually no color change under the same conditions. Figure 1 shows the response in the

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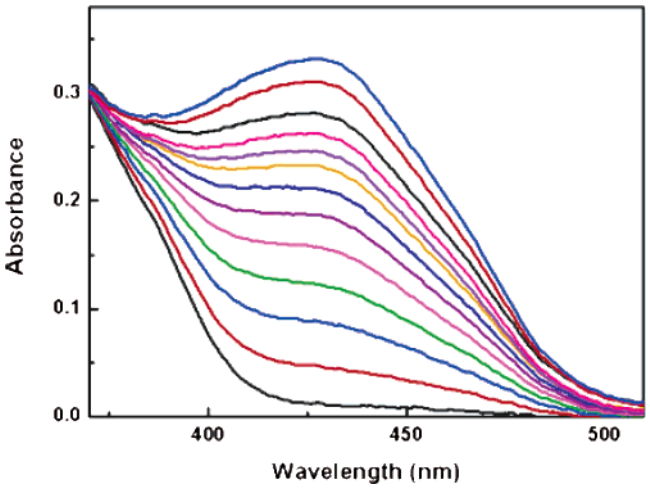

FIGURE 1. Ultraviolet-visible titration of **2** (100 μM) with 1,3-diaminopropane (0−120 mmol, bottom to top) in acetonitrile at 20 °C.

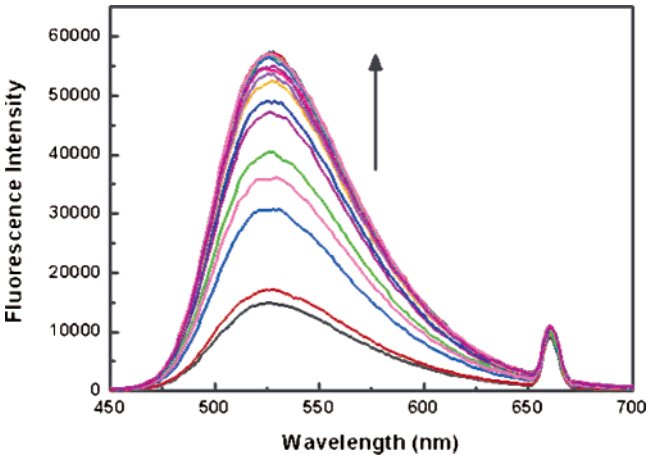

FIGURE 2. Fluorescence excitation titration of **2** (10 μM) with 1,3-diaminopropane (0−110 μM, bottom to top) in acetonitrile at 20 °C.

visible region of **2** to titration by 1,3-diaminopropane, illustrating both the wavelength shift and the enhanced intensity. In their fluorescence spectra, both **1** and **2** exhibit very small shifts upon complexation with aliphatic amines. Although the wavelengths are similar, at 515 nm for **1** and at 527 nm for **2**, the intensity is much strengthened for **2**. Figure 2 gives the response in the fluorescence excitation spectrum of **2** to titration by 1,3-diaminopropane, illustrating the strong increase in intensity without much change in wavelength. The greater extinction coefficient for the demethylated dye **2** suggests that the phenolic hydroxyl stabilizes the complex, making **2** a more sensitive chemosensor. The fluorescence maxima of the Jiang et al. molecule (eq 1) at 427 and 508 nm also do not shift on addition of 1,3-diaminopropane.

Binding constants were measured from the fluorescence spectra for a wide variety of amines (**3**−**48**, Chart 1), including aliphatic monoamines (primary, secondary, and tertiary), aliphatic diamines, cyclic amines, anilines, and aromatics. Job's plots for both monoamines and diamines in Supporting Information indicated that the complexes have a 1:1 stoichiometry. The limit to the detection of the diamines by **2** was at the ppm level. Table 1 contains the binding constants and acidity information, when available, for all amines studied, and Figure 3 summarizes the binding results.

Discussion

Binding Mechanism. Figure 3 clearly illustrates the ability of **2** to bind with amines with considerable selectivity, as discussed in the next section. The existence of that binding does not depend on the choice of mechanism. There are several mechanistic variations of a basic binding scheme between **2** (represented as Ar−OH) and neutral amine bases (represented as B:).

Binding scheme A (excited-state proton transfer):

$$\mathrm{Ar{-}OH} \xrightarrow{h\nu} [\mathrm{Ar{-}OH}]^* \xrightarrow{\mathrm{B:}} [\mathrm{Ar{-}O^-BH^+}]^*$$

Binding scheme B (ground-state proton transfer):

$$\mathrm{Ar{-}OH} \overset{\mathrm{B:^-}}{\rightleftharpoons} \mathrm{Ar{-}O^-BH^+} \xrightarrow{h\nu} [\mathrm{Ar{-}O^-BH^+}]^*$$

Binding scheme C (ground-state hydrogen bonding):

$$\mathrm{Ar{-}OH} \overset{\mathrm{B:}}{\rightleftharpoons} \mathrm{Ar{-}OH\cdots B} \rightleftharpoons \mathrm{Ar{-}O^-\cdots HB^+} \xrightarrow{h\nu} [\mathrm{Ar{-}O^-\cdots HB^+}]^*$$

The complexes are represented in the first two schemes as ion pairs and in the last scheme as hydrogen bonded.

Chemosensor **2** contains three distinct active hydrogens. Naphtholic protons have aqueous pK_a values of about 9.2 and pK_a* values of about 2.8.[30] The respective figures for phenolic protons are 9.8 and 6.[37] In **2**, the naphtholate ion, but not the phenolate ion, is further stabilized by resonance with the anilide carbonyl group. Amidic protons exhibit a pK_a of 15−19 and should not be involved in any ionization process. The naphtholic proton is slightly more acidic than the phenolic proton in the ground state but more so in the excited state. It is clear from Figures 1 and 2 that **2** is sensitive to the presence of amine bases in both the ground and the excited states. In the absence of an amine, the solution is colorless, but on the addition of an amine in millimolar levels, a maximum appears in the visible spectrum at 427 nm (Figure 1), causing the solution to become light yellow. Addition of an amine at micromolar levels increases the peak at 537 nm in the fluorescence spectrum by a factor of about six. Thus, there are interactions between **2** and the amine in both the ground and the excited states, but the effect is far more intense in the excited state. For this reason, we made all binding measurements with the fluorescence spectra. Job's plots (Supporting Information) were measured from the ultraviolet/visible spectra for ethylenediamine and propylamine and found to indicate 1:1 complexes in both cases.

These results can be discussed in terms of any of the above three binding schemes. Jiang and co-workers[27] favored the excited-state proton transfer in their studies of anion binding. Such a phenomenon is well precedented in the literature.[31] In polar solvents, such as acetonitrile, ion pairs have been favored over hydrogen-bonded species.[34] Naphthols have been found to hydrogen bond with amines in the ground state, rather than lose a proton,[32] because of the relatively low acidity. Literature precedent, therefore, suggests that the phenomena in Figure 1 are the result of hydrogen bonding (binding scheme C), but those in Figure 2 are the result of excited-state proton transfer (binding scheme A).

Further information can be obtained by examining the ultraviolet/visible spectrum of **2** in the presence of a strong base,

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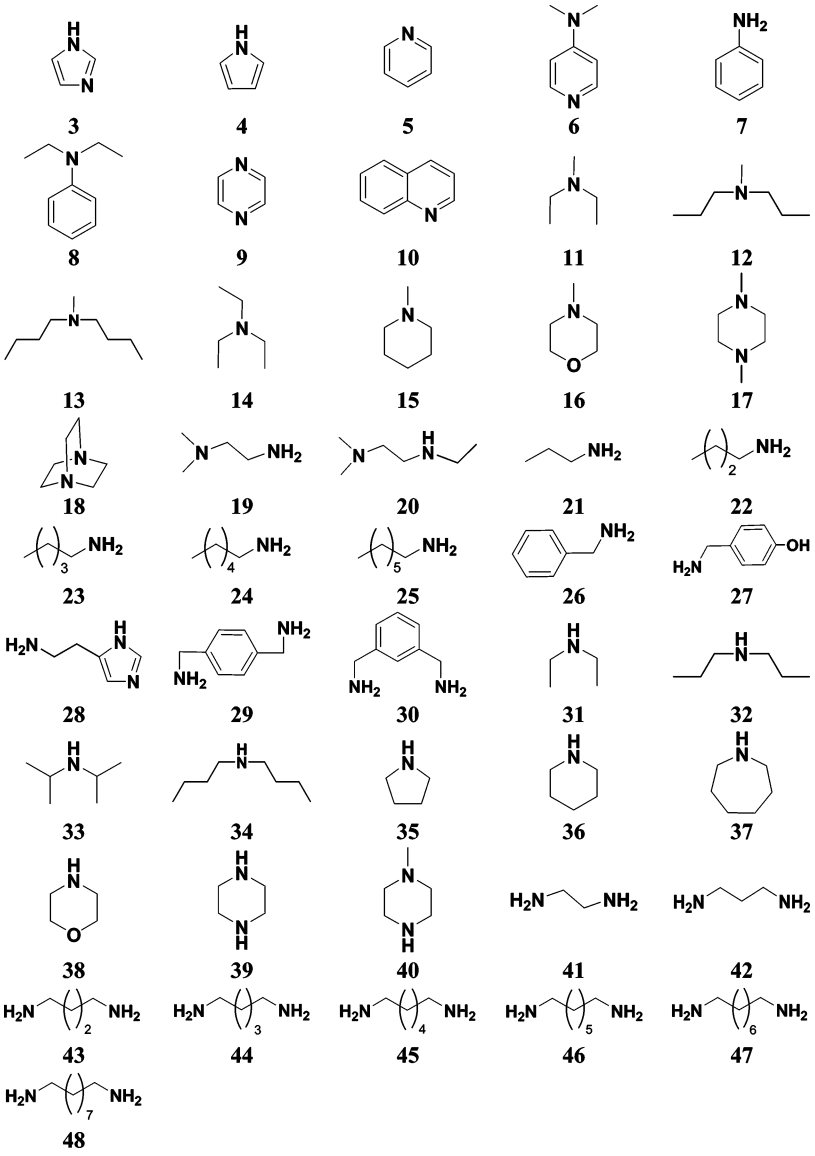
CHART 1. Amine Substrates for Detection



which would convert the chemosensor entirely to its conjugate base, simulating binding scheme B. The titration of **2** with tetramethylammonium hydroxide resulted in a single, broad transition in both the ground state (λ_{max} = 427 nm) and the excited state (λ_{max} = 527 nm; Figure 4). These results again illustrate the much stronger intensity of the effect in the excited state (note the concentration difference in the plots in Figure 4). The absence of sigmoidal behavior at low concentration results from the immediate removal of a proton at the lowest concentrations. As the concentration of base is increased, there is a change in the wavelength of the absorption, from 427 to 436 nm. The latter value corresponds to absorption from the fully deprotonated naphtholate ion, Ar−O$^-$, as in binding scheme B. In all our solutions of **2** in the presence of amine bases, the absorption remains at 427 nm. We conclude that the amines do not convert **2** to its conjugate base under our conditions, as expected for an acid with a pK_a of about 9.

One other point is worth making from Figure 4. The ground-state acidities of naphthols and phenols are nearly identical, so that double ionization of both hydroxyls in **2** would be masked in the titration plot. The removal of two protons with appreciably different acidities would produce two transitions separated by a plateau or inflection. The excited-state titration plot in Figure 4, corresponding to conditions under which the phenolic and naphtholic acidities are clearly different, is broader than the ground-state plot but also shows a single transition. Thus, double ionization is unlikely in the excited-state process.

We believe we can eliminate binding scheme B because the ultraviolet spectrum does not indicate complete ionization of **2** in the ground state in the presence of amines. We have no way of assessing what the wavelength shift would be with a hydrogen-bonded complex (binding scheme C). Prior work indicates that in acetonitrile the excited-state complexes involve ion pairs rather than hydrogen-bonded species.[27,31,34] The addition of water to the solution of the pre-existing complex resulted in only a minor loss of fluorescence intensity. Moreover, we used undried acetonitrile so that some water was present in all experiments and did not inhibit proton transfer. These observations support the hypothesis that the excited-state complexes are ion pairs between naphtholate and ammonium ions (binding scheme A) rather than nonionized hydrogen-bonded entities (binding scheme C), as water also could provide the hydrogen bonds.[31] Moreover, the lower basicity of water prevents it from supplanting the amines in the complex.

TABLE 1. Binding Constants and pK_a Values of Some Amines in Acetonitrile and Water

	amine	binding constant	pK_a (ACN)	pK_a (H_2O)[a]		amine	binding constant	pK_a (ACN)	pK_a (H_2O)[a]
3	imidazole	<10		7.09	**26**	benzylamine	7000	16.76[c]	9.33
4	pyrrole	<10		16.5	**27**	tyramine	19 000		9.22
5	pyridine	<10	12.33[b]	5.21	**28**	histamine	35 000		9.80, 5.94
6	4-(dimethylamino)pyridine	6900	17.74[b]	9.7	**29**	*p*-xylyldiamine	10 000		
7	aniline	<10	10.56[c]	4.60	**30**	*m*-xylyldiamine	26 000		
8	*N*,*N*-diethylaniline	<10		6.61	**31**	diethylamine	150 000	18.75[c]	11.09
9	pyrazine	<10		1.1	**32**	dipropylamine	50 000		11.0
10	quinoline	<10		4.9	**33**	diisopropylamine	150 000		11.13
11	*N*,*N*-diethylmethylamine	49 000		10.46	**34**	dibutylamine	61 000	18.31[c]	11.25
12	*N*,*N*-dipropyllmethylamine	28 000			**35**	pyrrolidine	110 000	19.58[c]	11.27
13	*N*,*N*-dibutylmethylamine	19 000			**36**	piperidine	180 000	18.92[c]	11.28
14	triethylamine	28 000	18.46[c]	10.75	**37**	hexamethyleneimine	33 000		
15	*N*-methylpiperidine	24 000		10.08	**38**	morpholine	200 000	16.61[c]	8.33
16	*N*-methylmorpholine	<10	15.59[d]	7.38	**39**	piperazine	63 000		9.83, 5.56
17	*N*,*N*′-dimethylpiperazine	6500			**40**	*N*-methylpiperazine	74 000		
18	DABCO	24 000	10.16[c]		**41**	1,2-diaminoethane	160 000	13.01[c]	6.99, 10.08
19	*N*,*N*-dimethylethylenediamine	40 000		6.63, 9.53	**42**	1,3-diaminopropane	180 000	14.98[c]	8.64, 10.62
20	*N*,*N*-dimethyl-*N*′ethylethylenediamine	42 000			**43**	1,4-diaminobutane	160 000	15.34[c]	9.35, 10.80
21	propylamine	80 000	18.22[c]	10.69	**44**	1,5-diaminopentane	290 000	16.97[c]	9.13, 10.25
22	butylamine	92 000	18.26[c]	10.66	**45**	1,6-diaminohexane	130 000		9.83, 10.93
23	amylamine	52 000		10.64	**46**	1,7-diaminoheptane	290 000		
24	hexylamine	60 000		10.64	**47**	1,8-diaminooctane	310 000		11.0, 10.1
25	heptylamine	29 000		10.66	**48**	1,9-diaminononane	86 000		

[a] Data taken from Perrin, D. D. *Dissociation Constants of Organic Bases in Aqueous Solution*; Butterworths: London, U.K., 1965. [b] Kaljurand, I.; Rodima, T.; Leito, I. *J. Org. Chem.* **2000**, *65*, 6202−6208. [c] Reference 32. [d] Beltrame, P.; Gelli, G.; Loi, A. *Gazz. Chim. Ital.* **1980**, *110*, 491−494.

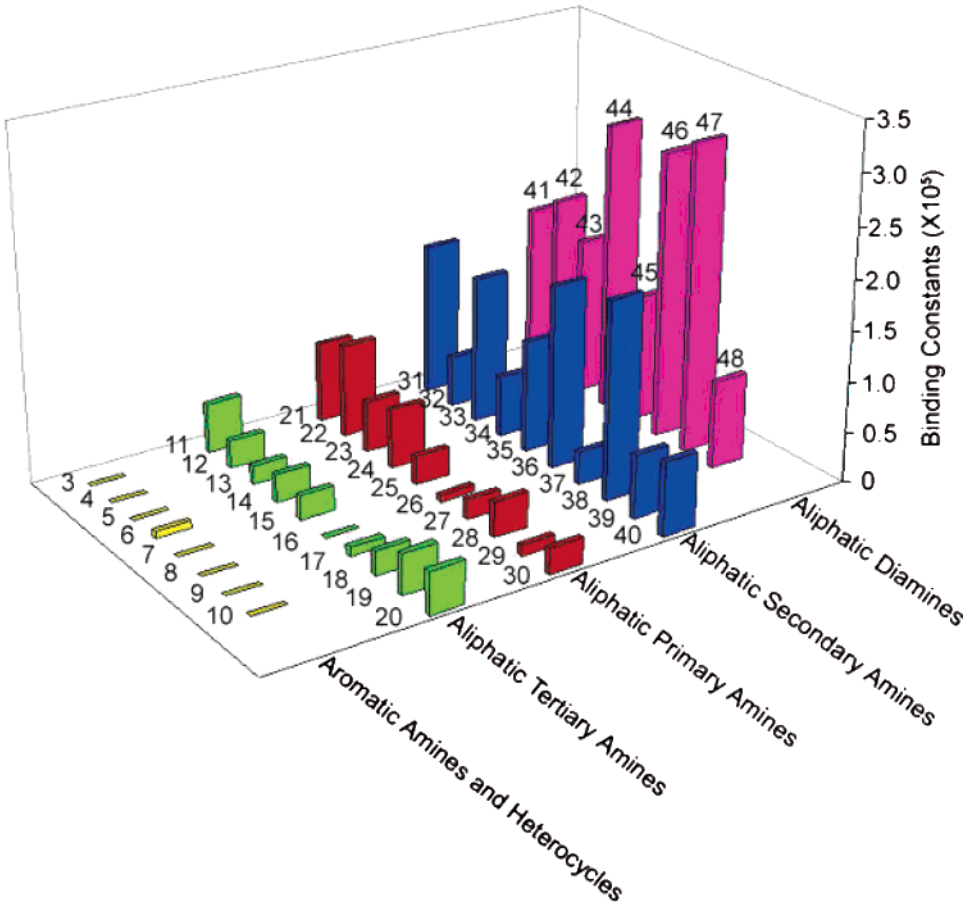


FIGURE 3. Binding profile of **2** with amines in acetonitrile.

Our binding studies were carried out in acetonitrile, which readily solvates monocations but not dications.[38] The lower dielectric constant of acetonitrile, in comparison with water, generally prohibits the formation of both dications and dianions. Thus, dissociation of the phenolic proton in the presence of the dissociated naphtholic anion is discouraged. By the same token, doubly protonated diamines are prohibited in acetonitrile. Consequently, the diamines formed 1:1 rather than 2:1 com-

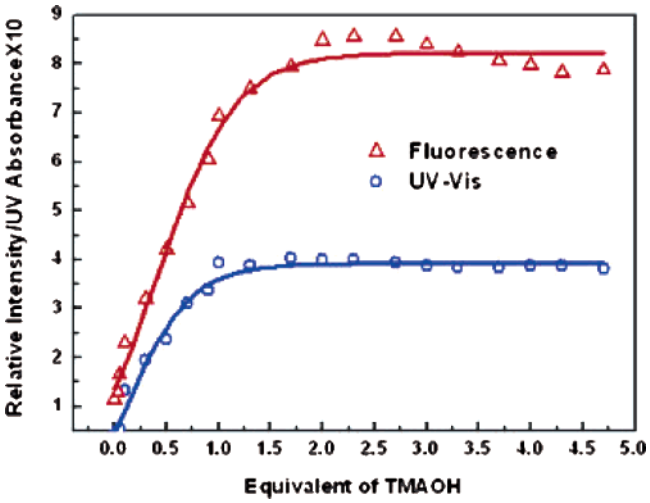
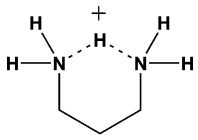


FIGURE 4. Titration of **2** in CH_3CN with tetramethylammonium hydroxide. In the fluorescence titration, the concentration of the sensor is 10 μM. In the UV−vis titration, the concentration is 100 μM.

plexes, as indicated by the Job's plot. Because monoprotonated, flexible diamines usually form strong intramolecular hydrogen bonding, structures such as **49** are the likely ammonium component of the complex for flexible diamines.[38,39]



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Although we have favored binding scheme A, we hasten to emphasize that the evidence is not overwhelming. The details of the mechanism, however, are not important to the efficacy of **2** as a chemosensor for amines, which is the main point of this study.

NMR titrations (Figure 5) show distinct NH/OH proton resonances in the absence of the amine (bottom spectrum). The resonances disappear with as little as 0.1 equiv of the amine present. These observations, however, are subject to several interpretations. The interactions represent kinetic rather than thermodynamic effects and could be intermolecular. About all we can say is that all the acidic protons are exchanging rapidly on the NMR time scale.

Finally, the binding ability of Jiang's molecule (eq 1) with 1,3-diaminopropane was found to be 9400, in comparison with 180 000 for **2**. The molecules differ in the bromine atom on the naphthol ring and the hydroxy group on the phenyl ring. It is doubtful that the bromine atom plays any role in the binding process. Therefore, the hydroxy group must enhance binding in some way, most likely by hydrogen bonds with the amine substrate, as illustrated in the next section.

Amine Selectivity. The arbitrary structural distinctions between nitrogen and oxygen functionalities are important to note in this context. Whereas there are primary ($R-NH_2$), secondary (RR′NH), and tertiary (RR′R″N) amines, alcohols (R−OH) are considered distinct functional groups from ethers (RR′O). On the other hand, whereas the aromatic congeners of alcohols (Ar−OH) are called phenols and never aromatic alcohols, anilines ($Ar-NH_2$) still are considered to be aromatic amines. There is no neutral oxygen analogue to trigonal amines such as pyridine, but pyrroles have an analogue in furans. For practical reasons, in this study we make a threefold categorization of amines: (1) simple, unconjugated aliphatic amines (both cyclic and acyclic), (2) anilines and other conjugated amines, and (3) aromatics (pyridines and pyrroles). We exclude carbonyl derivatives (amides) from this study, as they would have very low binding to **2**. Because of the general basic properties of amines, all these molecules can form positively charged onium salts by either protonation or alkylation.

A glance at Figure 3 and Table 1 indicates that the major selectivity is between aliphatic amines and the other two categories. For almost all aromatic (pyridine, pyrrole) and conjugated (aniline) substrates, the binding constants were unobservably small (<10). This selectivity results largely from the difference in basicity between saturated (aliphatic) and unsaturated (aromatic, anilinic) amines. Table 1 has numerous pK_a values in water and several in acetonitrile for the conjugate acids of the amines. Acidities are much lower in acetonitrile (and, hence, the amines are stronger bases) than in water, variably but by roughly 6−8 orders of magnitude. Hall reported an approximately linear relationship between acidities in the two solvents.[40,41]

There are several reasons for the lower basicities of aromatic and unconjugated amines in comparison with aliphatic amines. In pyridines, the acidic proton is on a trigonal nitrogen (protons on trigonal nitrogen or carbon always have heightened acidity in comparison with those of the analogous tetragonal systems, so the conjugate base is less basic). Anilines have low basicity because the nitrogen lone pair is involved in delocalization with the aromatic ring. These substrates then are less able to form the excited-state complex through proton transfer. The one apparent exception is 4-(dimethylamino)pyridine (**6**), which binds weakly. Strong electron donation from the 4-dimethylamino group, however, brings its basicity into the range of aliphatic amines (Table 1). Pyrrole (**4**) protonates on carbon rather than nitrogen, confirming the very low basicity of the lone pair on nitrogen, which is part of the aromatic sextet. The figure in Table 1 for pyrrole, therefore, reflects carbon rather than nitrogen acidity. Protonated pyrrole, lacking the ^+N-H bonds of the other systems, binds poorly to ionized **2**.

Although amine basicity is the primary factor in determining binding, other factors also are important and indeed are interlocking: (1) hydrogen bonding, particularly by other amine protons, (2) steric and conformational effects, (3) resonance, and (4) induction. We already have seen the interplay of resonance effects in 4-(dimethylamino)pyridine, of hydrogen bonding (the lack thereof) in pyrrole, and of induction from sp^2 hybridization in pyridines as factors influencing basicity. These factors also have secondary influences within the set of aliphatic amines. Low basicity prevents many other functionalities, including alcohols, ethers, and carbonyl compounds, from being detected by **2**.

The second, third, and fourth sets in Figure 3 represent the aliphatic monoamines, all of which bind to varying degrees. On average, secondary amines exhibit the strongest binding, followed by primary amines, and then by tertiary amines. The

(38) Coetzee, J. F.; Padmanabhan, G. R. *J. Am. Chem. Soc.* **1965**, *87*, 5005−5010.
(39) Aue, D. H.; Webb, H. M.; Bowers, M. T. *J. Am. Chem. Soc.* **1973**, *95*, 2699−2701.
(40) Hall, H. K. *J. Am. Chem. Soc.* **1957**, *79*, 5441−5444.
(41) Hall, H. K. *J. Phys. Chem.* **1956**, *60*, 63−70.

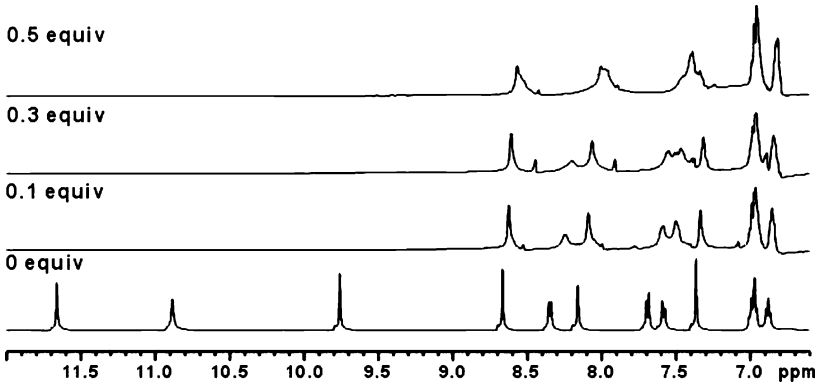
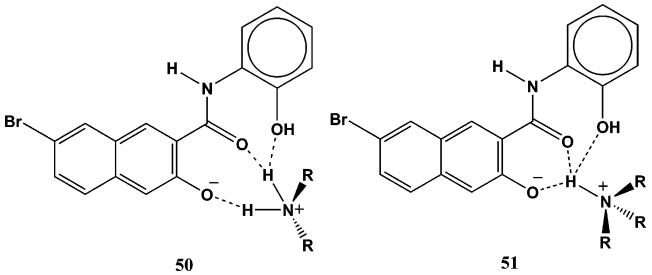


FIGURE 5. NMR titration of 0.1 M **2** with ethylenediamine in CD_3CN containing 20% (v/v) DMSO-d_6 at 20 °C.

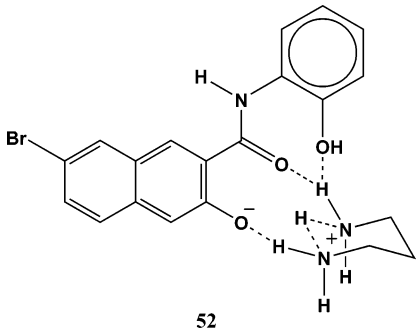
acidity data in Table 1 confirm that secondary amines indeed are the strongest bases on average. The reasons are complex and numerous, including induction by alkyl groups (favoring tertiary over secondary over primary), hydrogen bonding (favoring primary over secondary over tertiary), and steric effects (favoring primary over secondary over tertiary). Hall emphasized the importance of hydration/dehydration of the neutral amine and its conjugate acid.[40] The net result is the higher basicity of the secondary amines.

In addition and linked to the basicity effect, hydrogen bonding in the excited-state complex may play an important role. Given a singly ionized naphthylate system, the negative charge is delocalized through the aromatic ring to the amide carbonyl group. This dicarbonyl system, analogous to acetylacetonate, can serve as a strong hydrogen-bond acceptor. Secondary amines are the ideal donor, as in **50**, in which both the just-transferred and the originally existing protons are hydrogen bonded. The two $^+$N−H protons each may bond with a negatively charged carbonyl oxygen. In the ideal geometry for complex **50**, the two oxygens and the two hydrogens lie in a single plane. As a result, the third hydrogen of the conjugate acid of a primary amine (one of the R groups in **50**) is disposed out of this plane. In this geometry, it is unavailable for intramolecular hydrogen bonding and is less well-stabilized by the solvent, acetonitrile. The conjugate acids of tertiary amines offer only a single $^+$N−H group (the proton having just been transferred). It presumably is placed midway between the carbonyl oxygens, providing the thermodynamic stabilization of only one hydrogen bond (**51**). Thus, secondary amines have the optimal hydrogen-bonding opportunity. A number of amine pairs illustrate the lower binding of the tertiary systems in comparison with the secondary systems (Figure 3 and Table 1): diethyl (**31**/**11**), dipropyl (**32**/**12**), dibutyl (**34**/**13**), piperidine (**36**/**15**), morpholine (**38**/**16**), and *N*-methylpiperazine (**40**/**17**). In each case, the replacement of N−H by N−CH_3 significantly inhibits binding.



Additional solvation of the amine proton by the phenolic hydroxyl also is illustrated in **50** and **51**. The importance of such an interaction is reinforced by the much lower binding constant of Jiang's molecule (eq 1), which has H in place of OH on phenyl, than that of **2** (9400 vs 180 000). The fact that **2** also binds more strongly than **1** (with OMe in place of OH) may result from some steric inhibition of the phenolic interaction included in **50** and **51**.

There are additional, more idiosyncratic, effects. Diisopropylamine (**33**) binds three times more strongly than dipropylamine (**32**). The interlocked isopropyl groups may be less sterically constraining than the freer propyl groups. Dibutylamine (**34**) is similarly weak, and the cyclic (tied-back) amines (pyrrolidine, **35**; piperidine, **36**) generally are stronger. *N*-Methylmorpholine (**16**) is particularly low, even for a tertiary amine. The strongly electron-withdrawing oxygen at the four position increases the acidity of the conjugate acid substantially (Table 1) into the range of the aromatic amines, so the amine itself is a particularly weak base for an aliphatic amine. *N*,*N*-Dimethylpiperazine (**17**) exhibits a similar but smaller effect, as nitrogen is less electron-withdrawing than oxygen. Among the primary amines, the weakest binding materials are benzylamine (**26**) and tyramine (**27**), which also are the weakest bases (Table 1) presumably because of the electron-withdrawing effect of the aryl rings.



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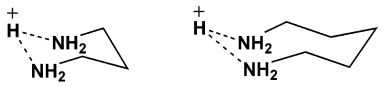
The doubly primary, flexible diamines are the best binding substrates (fifth set in Figure 3). We have suggested that the ammonium component of the excited-state complex resembles **49** for these diamines, with an intramolecular hydrogen bond. The peculiar geometry of such systems renders the possibility that three hydrogens are involved in binding, as in **52**. The two oxygens, the two nitrogens, and the three hydrogens may be close to a single plane. The half-tertiary, half-primary diamine **19** cannot provide such a hydrogen-bonding network and, hence, exhibits much-reduced binding (**19** and the related **20** are listed among the aliphatic tertiary amines in the second row of Figure

3). It is noted that only the doubly primary diamines, with an odd number of carbon atoms between the nitrogens, can offer the chair/crown conformation of **52**, as in **53**−**55** for molecules **42**, **44**, and **46**, respectively. These systems exhibit heightened binding in comparison with that of **43** and **45**. The amine basicities (conjugate acid acidities) follow this same trend (Table 1). The larger ring of **47** apparently permits an optimal conformation for hydrogen bonding, but entropic factors dominate in the longest system **49**. Constrained diamines such as **29**, **30**, and the piperazines (**17**, **18**, **39**, and **40**) sterically inhibit the close approach of the two nitrogen atoms and prohibit conformations such as **49**.



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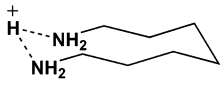

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Conclusions

Molecule **2** is a sensitive and specific chemosensor for aliphatic amines. It forms an excited-state complex that is readily detectable by fluorescence. Complexation depends on the excited-state acidity of the naphtholic proton of **2**, the presence of an adjacent phenolic hydroxyl, and the basicity of the amines. Aromatic and conjugated amines, in which the nitrogen atom is sp^2 hybridized, are not detected. Almost all saturated aliphatic amines are strongly detected, with differences dependent primarily on amine basicity and to some extent on hydrogen-bonding and steric properties within the excited-state complex.

Experimental

7-Bromo-3-hydroxy-2-naphth-*o*-hydroxyanilide (2), Demethylated Naphthol AS-BI.[42] A solution of BBr_3 (1 mL, 10.6 mmol) in 10 mL of anhydrous CH_2Cl_2 was added dropwise to **1** (1.0 g, 2.7 mmol; purchased from a commercial supplier) dissolved in 40 mL of anhydrous CH_2Cl_2 under N_2 at 0 °C. The resulting suspension was allowed to stir overnight at room temperature. The reaction was quenched by the dropwise addition of 50 mL of CH_3OH. The resulting mixture was stirred at room temperature for 2 h. The solvent was evaporated to dryness, 10 mL of CH_3OH was added, and evaporation was repeated. A total of 10 portions of CH_3OH (10 mL each) were added and evaporated in this fashion. The light gray solid then was dissolved in hot MeOH and precipitated with diethyl ether to yield 0.65 g (67%) of an off-white crystalline powder (**2**): ^{1}H NMR (DMSO-d_6) 11.03 (s, 1H), 10.41 (s, 1H), 9.19 (s, 1H), 8.12−6.33 (m, 9H); ^{13}C NMR (DMSO-d_6) 149.3, 146.2, 119.1, 118.2, 117.7, 115.1, 51.4, 33.8. Anal. Calcd for $C_{17}H_{12}BrNO_3$: C, 57.00; H, 3.38; N, 3.91. Found: C, 57.03; H, 3.43; N, 3.84.

(42) Rodgers, S. J.; Lee, C. W.; Ng, C. Y.; Raymond, K. N. *Inorg. Chem.* **1987**, *26*, 1622−1625.

Sample Preparation. Stock solutions of the chemosensor **2** and of the amines were prepared freshly just prior to use. The solution of **2** in acetonitrile changes from colorless to yellow after 2 days, probably a result of oxidation. For stoichiometry studies (Job's plots), nine samples were prepared with the molar ratio of **2** to the amine varying from 1:9 to 9:1. The total concentration of **2** and the amine was maintained at 0.1 M for all samples. For each fluorescence study, 16 samples were prepared. The concentration of the chemosensor **2** was maintained at 10 M throughout the experiment, while the concentration of the amine varied from 1 to 100 M.

Spectral Measurements. All measurements were done at 20±1 °C. A 1-cm cuvette was used for both UV/vis and fluorescent determinations. For Job's plots, the absorbance at 427 nm was monitored as a function of the molar ratio of **2** and the amines. In fluorescent measurements, an excitation wavelength of 335 nm was used for both **1** and **2**. In the binding studies, the emission at 512 nm for **1** and at 527 nm for **2** was monitored.

Curve Fitting. Quantitative treatment of the fluorescent data starts with the premise that the intensity is a sum of the contributions of all the fluorescent species in solution. For a system containing substrate S, ligand L, and complex SL with a binding stoichiometry of 1:1 (eq 2),

$$S + L \rightleftharpoons SL \tag{2}$$

as demonstrated by the Job's plots, the fluorescence intensity F is given by eq 3,

$$F = K_S \cdot [S] + K_{SL} \cdot [SL] + K_L \cdot [L] \tag{3}$$

in which the K_X represent the proportionality constants between the intensity and the concentration for each species. In this experiment, the amine substrate is not fluorescent ($K_S = 0$), and the total concentration of the host, $[L]_T$, is constant. In the absence of substrate, $[S] = [SL] = 0$, and in the presence of the sensor, with a total concentration of $[L]_T$, the initial fluorescence intensity, F_0, is given by eq 4.

$$F_0 = K_L \cdot [L]_T \tag{4}$$

Mass balance requires that $[L]_T = [L] + [SL]$, and the binding constant K is expressed by eq 5.

$$K = [SL]/([L][S]) \tag{5}$$

Combining eqs 3−5 and mass balance gives eq 6,

$$(F/F_0) = ((K_{SL}/K_L)K[S] + 1)/(K[S] + 1) \tag{6}$$

in which F is the fluorescence intensity. A plot of F/F_0 versus the substrate concentration, [S], was constructed, and the curve was fitted by nonlinear least-squares regression to give K.

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Supporting Information Available: Job's plots. This material is available free of charge via the Internet at http://pubs.acs.org.

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