JOC The Journal of Organic Chemistry

Article

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Thioureas as Reporting Elements for Metal-Responsive Fluorescent Chemosensors

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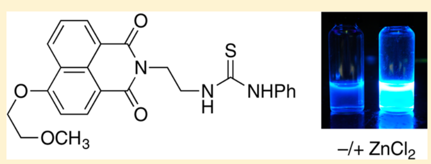
Ⓢ Supporting Information

ABSTRACT: Proof that sulfur is a viable reporting element for the development of fluorescent chemosensors for metal ions is presented. To date, the majority of metal-responsive fluorescent chemosensors have relied on metal−nitrogen coordination to provide a fluorescence response, most commonly by suppressing photoinduced electron transfer (PET) quenching. While chemosensors with direct application to biology, medicine, and analytical chemistry have been so developed, reliance on the coordination chemistry of nitrogen remains a practical and conceptual limitation. Building on the fact that thioureas can quench fluorescence emission by PET, it is shown that the quenched emission of thiourea-appended naphthalimides can be restored by metal binding and that metal affinity and selectivity can be controlled through structural modification of the thiourea substituents. Further, such chemosensors can function in aqueous media and, unlike nitrogen-based chemosensors, are unresponsive to increases in [H^+]. Given that the coordination properties of sulfur are distinct from those of nitrogen, this work lays the foundation for the development of a new class of interesting and useful metal-responsive fluorescent probes.



■ INTRODUCTION

Chemosensors that provide a fluorescent response to reversible metal ion binding have broad biological and environmental application.[1,2] One of the most general approaches to developing such fluorescent chemosensors relies on photoinduced electron transfer (PET) from an amine appended to a reporting fluorophore.[3,4] In the absence of metal ion, PET from the amine lone pair quenches fluorescence, and emission is restored upon metal coordination. Beyond the generality of the signaling mechanism, the appeal of the aminofluorophore motif stems from the fact that metal ion affinity and selectivity can be controlled by variation of the nitrogen subsitutents. However, reliance on nitrogen and its coordination properties for signaling is necessarily a limitation of this approach, and the validation of alternative reporting elements is clearly desirable.

Of the neighboring heteroatoms that might be considered as PET quenchers, oxygen is an insufficient donor and phosphorus undergoes ready oxidation.[5] While sulfur is a viable candidate, there are no well-defined examples of metal-responsive fluorescent chemosensors that rely directly on sulfur as a reporting element.[6−9] We describe here a well-defined thiourea-based system which functions by suppression of PET, in protic media and show that metal binding selectivity and affinity can be controlled by structural variation. We anticipate that this will facilitate the design and development of a range new fluorescent chemosensors.

Known Thiourea-Based Fluorescent Chemosensors. Thioureas have been used in the development of anion-responsive fluorescent chemosensors.[6] In such systems, hydrogen bonding of anions by thiourea N−H groups increases the electron density of the thiourea and enhances PET quenching of a proximate fluorophore, leading to reduction in fluorescence emission. These efforts are distinct from the present work in that they focus on anion- rather than cation-induced changes in emission, do not involve direct coordination of the thiourea sulfur atom, generally rely on fluorescence quenching rather than fluorescence enhancement for signaling, and have not been shown to function in hydroxylic/aqueous solvents.

■ RESULTS AND DISCUSSION

Thiourea/Fluorophore Conjugates Studied. A series of thioureas based on a naphthalimide fluorophore (**1**−**8**, Figure 1)[10] were prepared, the synthesis of **6** being representative

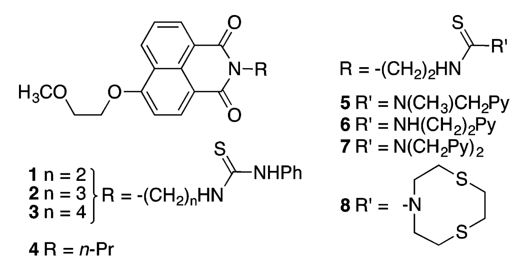


Figure 1. Thiourea-based chemosensors and control compounds (Py = 2-pyridyl).

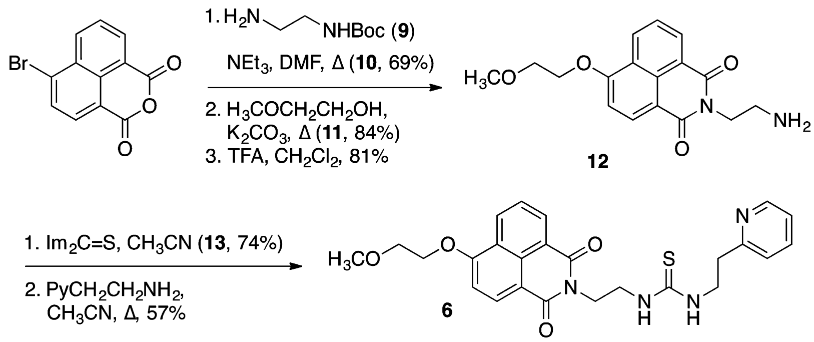
(Scheme 1). Phenylthioureas **1**−**3** vary in alkyl spacer length; **4** serves as an *N*-alkyl fluorophore control; and **5**−**8** bear side chains with additional metal-coordinating functionality.[11]

Optical Properties and Quenching Mechanism. Other than variation in quantum yield, the optical properties of **1**−**8** are essentially the same as those of the naphthalimide: substituent effects are minimal, and all compounds have

Received: February 14, 2013

dx.doi.org/10.1021/jo4003129 | J. Org. Chem. XXXX, XXX, XXX−XXX

Scheme 1. Synthesis of 6, a Representative Thiourea (Py = 2-Pyridyl, Im = Imidazolyl)[a]



[a]See the Experimental Section for full synthetic details.

identical absorption and emission maxima (Table 1). Thioureas **1−3** all show diminished fluorescence relative to control **4**, and emission intensity increases as a function of increased fluorophore/thiourea separation, as expected for PET quenching.[3] This distance dependence, and electrochemical measurements on **1** and **4**,[12] strongly support PET quenching of fluorescence emission in these thioureas.

Table 1. Relevant Optical Properties of 1−8[a]

	$\varepsilon/10^3$ M^{-1} cm^{-1}	ϕ^b		$\varepsilon/10^3$ $M^{-1}cm^{-1}$	ϕ^b
1	11.1	0.02	**5**	13.5	0.03
2	12.8	0.08	**6**	13.3	0.05
3	12.8	0.15	**7**	12.6	0.05
4	13.3	0.92	**8**	13.0	0.03

[a]All spectra measured in CH_3OH. Emission spectra acquired at 3.3 μM. Longest λ absorption/excitation maxima 367 nm; emission maxima 446 nm. [b]Quantum yields relative to anthracene (ϕ = 0.30).

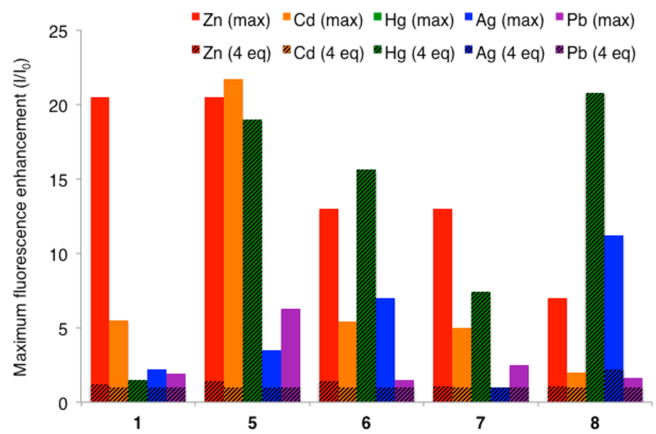


Figure 2. Maximum metal-induced ratiometric fluorescence enhancement of **1** and **5−8** (3.3 μM in CH_3OH; λ_{em} = 446 nm). Ratiometric enhancement in the presence of 4 equiv of metal ion shown by cross-hatching to emphasize Hg^{2+} selectivity. See Tables 2 and 3.

Metal Ion Response in Methanol. Methanolic solutions of thioureas **1** and **5−8** were titrated with $ZnCl_2$, $HgCl_2$, $CdCl_2$, $AgClO_4$, and $Pb(NO_3)_2$ (see the Experimental Section). The intrinsic binding preferences of the thiourea fragment are illustrated by **1**, which shows a strong, low-affinity response to Zn^{2+}, a weak response to Cd^{2+}, and no significant response to the other ions (Figure 2, Tables 2 and 3).[13] Titrations with alkaline and alkaline earth metal salts lead to no change in fluorescence emission.

The affinity and selectivity of the metal response can be dramatically altered by extension with known metal coordinating groups, in the present case alkylpyridines or a thiacrown ether (Figure 2, Tables 2 and 3). The titration of **5** in CH_3OH with $ZnCl_2$ is representative in showing a continuous increase in fluorescence emission up to a maximum I/I_0 (Figure 3),[14] after which emission remains constant. Plots of I/I_0 vs log[metal ion] are consistent with simple saturation binding, and nonlinear least-squares fitting allows ready extraction of apparent first-order K_d values (Table 2);[15,16] titrations of **1** and **5−7** with $ZnCl_2$ are representative (Figure 4). Metal binding is reversible,[17] and importantly, fluorescence responses are insensitive to the addition of excess acid (TFA).

Methanolic Metal Ion Titrations. Several aspects of these data provide insight for the further development and use of thioureas in fluorescent chemosensors. First, as titrations of **1** indicate, the thiourea itself is not a strongly coordinating ligand, even for thiophilic metals. However, it is clearly a viable reporting element for metal detection: the maximum ratiometric fluorescence enhancements, $(I/I_0)_{max}$, of up to ~20-fold represent a significant dynamic range; the associated maximum effective quantum yields ($\phi_{max} = \phi_{initial} \times (I/I_0)_{max}$) of up to 0.6−0.7 indicate that substantial recovery of quenched emission is possible (Figure 2, Table 3; compare to **4**, ϕ = 0.92).

Table 2. Apparent log(K_d) (M) for Titrations of 1 and 5−8[a,b]

	Zn^{2+}	Cd^{2+}	Hg^{2+}	Ag^+	Pb^{2+}
1	−1.3	−1.6	-	-	-
5	−3.2	−3.2	−5.8	-	−2.5
6	−2.1	−2.3	−5.3	−2.3	-
7	−2.9	−3.1	−6.1	-	-
8	−1.2	-	−5.2	−4.4	-

[a]Titrations in CH_3OH at 3.3 μM chemosensor. [b]Entries marked "-" indicate binding too weak to allow K_d determination.

Second, the identity of the additional metal-coordinating group(s) determines the metal-binding affinity and selectivity. The highest affinities observed in **5−8** are for Hg^{2+}, and **5−8** are all highly Hg^{2+}-selective chemosensors (Figure 1, Table 2). Their apparent affinities lie at the limit of determination in the present system.[18] (As our routine measurements must be made at micromolar chemosensor concentrations, this represents the lower limit of the K_d values we can determine; brighter probes suitable for use at lower concentration will be required to determine actual K_d values fluorimetrically.) The variations in Zn^{2+} affinities of **5−8** are instructive. They indicate that only

Table 3. Values of $(I/I_0)_{max}$ and (ϕ_{max}) for Titrations of **1** and **5**−**8**[a,b,c]

	Zn^{2+}	Cd^{2+}	Hg^{2+}	Ag^{+}	Pb^{2+}
1	20.5 (0.41)	5.5 (0.11)	-	-	-
5	20.5 (0.62)	21.7 (0.65)	19 (0.57)	-	6.3 (0.19)
6	13 (0.65)	5.4 (0.27)	15.6 (0.78)	7 (0.35)	-
7	13 (0.65)	5 (0.25)	7.4 (0.37)	-	-
8	7 (0.21)	-	20.8 (0.62)	11.2 (0.33)	-

[a]Maximum observed I/I_0 as shown in Figure 2. [b]$\phi_{max} = \phi_{initial} \times (I/I_0)_{max}$. ϕ_{max} in parentheses. [c]Entries marked "-" indicate that saturated binding, and thus I/I_0(max), were not reached.

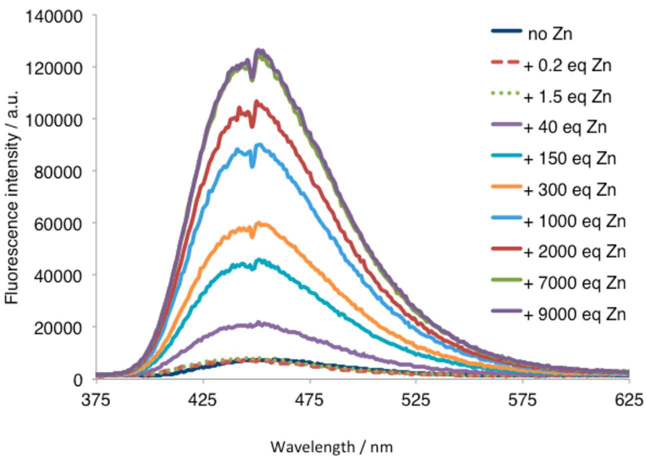


Figure 3. Titration of **5** (3.3 μM in CH_3OH) with $ZnCl_2$.

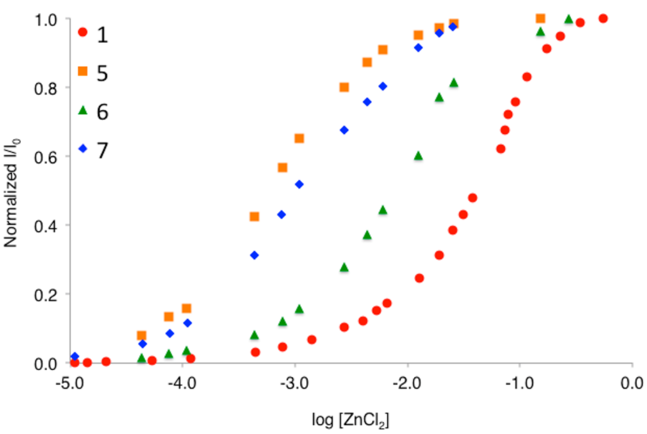


Figure 4. Binding curves from titration of **1** and **5**−**7** with $ZnCl_2$.

one of the *N*-substituents on the thiourea contributes to metal binding (**5** vs **7**), reflecting the planarity of the thiourea N resulting from conjugation to the thiocarbonyl,[19] and that even small changes in the relative orientation of the coordinating heteroatom lone pairs have a significant impact on metal affinity (**5** vs **6**).[20]

Third, while there is significant variation in $(I/I_0)_{max}$, there is no clear correlation with metal ion identity. The difference in $(I/I_0)_{max}$ for **1**·Zn^{2+} and **1**·Cd^{2+} indicates that coordinated metals do not have an equal intrinsic impact on PET quenching. That the presence of an additional ligand can increase $(I/I_0)_{max}$ (**1**·Cd^{2+} vs **5**·Cd^{2+}) indicates that details of the metal coordination environment beyond thiourea binding must also play a role in the fluorescence response. This sensitivity to structural variation holds promise for fine control of fluorescence response in future chemosensors.

Finally, it is instructive to consider the differences in behavior between **5** and **7**. These ligands exhibit nearly identical K_d values for a given metal (Zn, Cd, or Hg) and presumably provide nearly identical coordination environments. However, $(I/I_0)_{max}$ is consistently lower for complexes of **7** than **5**, indicating the influence of additional factors. We propose that one of these factors is the position of the *s-cis*/*s-trans* conformational equilibrium of nonemissive metal-complexed species in which thiourea is not ligated (Figure 5). The

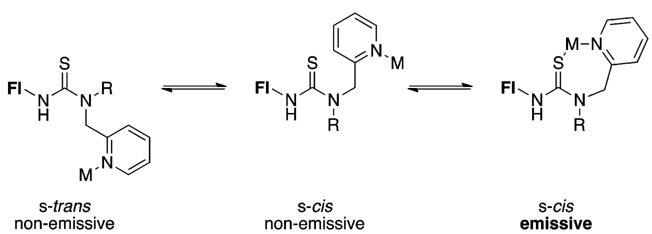


Figure 5. Minimal form of the *s-cis*/*s-trans* equilibria in metal complexes of **5** and **7** (**Fl** = naphthalimide fragment; M = metal ion; R = CH_3 (**5**), CH_2Py (**7**)).

presence of such species is consistent with the low intrinsic affinity of the thiourea fragment for metal ions. In **5**·M (Figure 5, R = CH_3), the *s-cis* isomer should be favored over the *s-trans* isomer for steric reasons,[21] while in **7**·M the isomer ratio should be closer to unity (Figure 5, R = CH_2Py).[22] An increase in the relative population of the *s-cis* conformation should be accompanied by an increase in the population of the emissive thiourea-coordinated state, increasing the observed $(I/I_0)_{max}$. This would thus account for the fluorescence enhancements of **5** being larger than those of **7**.

If correct, this provides an important guideline for the development of future thiourea chemosensors: monosubstituted or conformationally constrained analogues should exhibit the largest increases in fluorescence upon metal binding by favoring the *s-cis* conformation.

Metal Ion Response in Aqueous Media. The solubility of **5**−**8** in H_2O is not sufficient to prepare the requisite micromolar solutions by serial dilution in pure water. However, dilution of methanolic stock solutions allows preparation of suitable aqueous samples. Titrations of **5**−**7** carried out in 9:1 H_2O/CH_3OH reveal promising and useful information (Table 4).

Although the dipicolylamine moiety is known to have high affinity for aqueous Zn^{2+} and Cd^{2+}, as noted above, the conjugation of the amine nitrogen with the thiocarbonyl precludes simultaneous engagement of both pyridine fragments and coordination of the thiourea. The diminished aqueous affinity of **5**−**7** for these metals is thus not surprising. The strong response of **5** to Zn^{2+} ($(I/I_0)_{max}$ = 9) indicates that thiourea-based signaling functions effectively in aqueous

dx.doi.org/10.1021/jo4003129 | J. Org. Chem. XXXX, XXX, XXX−XXX

Table 4. Apparent $\log(K_d)$ (M) and $((I/I_0)_{max})$ for Aqueous Titrations of 5−7[a,b]

	Zn^{2+}	Cd^{2+}	Hg^{2+}	Ag^+	Pb^{2+}
5	−1.1 (9.3)	−2.1 (4.6)	−5.9 (4.4)	-	-
6	−0.4[c] (5.0)	−1.7 (3.0)	−5.8 (6.8)	-	-
7	−0.9 (5.7)	−2.5 (4.5)	−6.0 (8.6)	-	-
7			−7.8 (8.3)[d]		

[a]Titrations in 9:1 H_2O/CH_3OH at 3.3 μM chemosensor. [b]Entries marked "-" indicate binding too weak to allow K_d determination. [c]$\log(K_d)$ extrapolated; maximum response could not be reached. [d]Measured at 33 nM chemosensor. See text and ref 23.

solution and that alternate chemosensor architectures should permit the development of higher affinity Zn^{2+} and Cd^{2+} probes. It is noteworthy that, as in pure CH_3OH, the addition of excess acid (TFA) does not alter fluorescence emission, underscoring this advantage of thiourea-based fluorescent probes relative to the majority of known nitrogen-based systems.

The high affinity of **5**−**7** for Hg^{2+} is retained in aqueous media, with $\log(K_d)$ values at or near the limits of determination. The maximum observed fluorescent enhancements of **5** and **6** are diminished relative to titrations in CH_3OH, but the maximum enhancement for **7** ($(I/I_0)_{max}$ = 8.5) actually increases slightly. The naphthalimide chromophore is not sufficiently absorptive to allow routine titration at probe concentrations much below the micromolar level, although results with 100-fold more dilute solutions ([**7**] = 33 nM) indicate that $\log(K_d)$ for **7**·Hg^{2+} is at least −7.8.[23] These data suggest that variation of the reporting fluorophore, without further modification of the metal-binding domain could readily lead to selective fluorescence detection of aqueous Hg^{2+} at analytically useful concentrations,[18] especially as there is no response to the potential competing ions Ag^+ and Pb^{2+}.

CONCLUSIONS

This work demonstrates that modulation of PET quenching by thioureas provides a basis for the development of metal-responsive fluorescent chemosensors in protic media. This is significant in that most known metal-responsive chemosensors rely on binding to a nitrogen atom, and the coordination chemistry of nitrogen has dominated fluorescent chemosensor development to date. Given its distinct coordination preferences, the addition of sulfur to the group of viable reporting heteroatoms is significant expansion. In addition, unlike amine-based fluorescent probes, the thioureas reported here do not to respond to the addition of acid, indicating that they will be less susceptible to pH-dependent variation in emission. We expect these findings will have an impact beyond the present work.

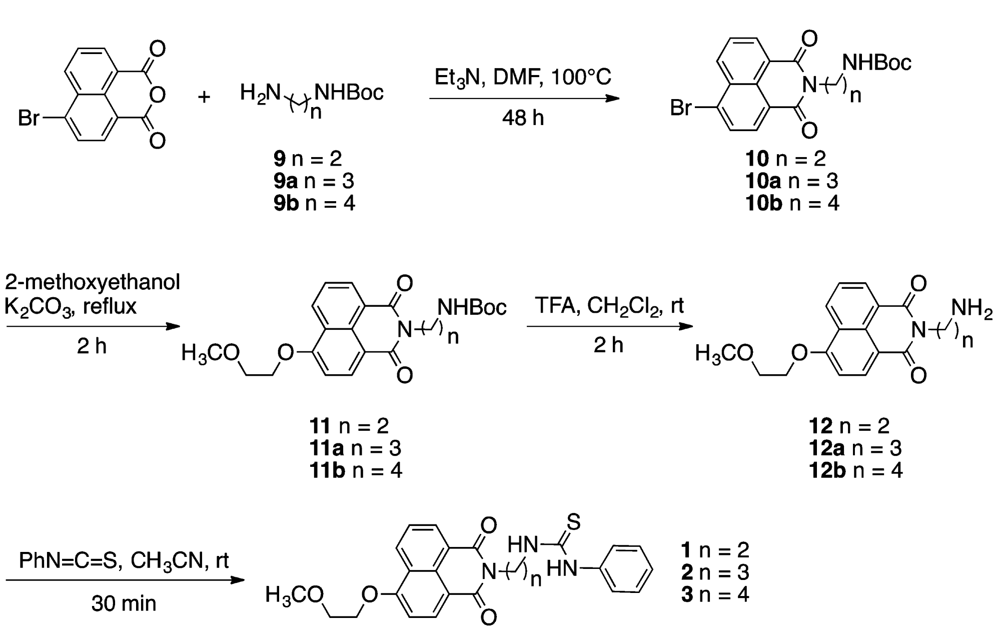
It is anticipated that further variation of binding domain and fluorophore will lead to the development of practical probes for detection and visualization of aqueous Zn^{2+} and Hg^{2+}. To this end, we are currently preparing water-soluble congeners of **5**−**8** that differ in the orientation and spacing of the chelating groups relative to the thiourea reporter as well as exploring probes based on other recognition elements and evaluating more strongly absorptive fluorophores.

EXPERIMENTAL SECTION

General Methods. Synthetic procedures were carried out under an inert atmosphere, in dry solvent, using standard Schlenk techniques, unless otherwise noted. All reagents and solvents were reagent grade and were used without further purification unless otherwise specified. Flash chromatographic purification was performed using silica gel Merck 60 (particle size 0.040−0.063 mm), deactivated (20% triethylamine in hexane) silica gel Merck 60 (particle size 0.040−0.063 mm), or deactivated (5% water by weight) neutral aluminum oxide Sigma-Aldrich, Brockmann I, packed in glass columns; eluting solvent for each purification was determined by thin layer chromatography (TLC). Analytical thin-layer chromatography was performed using Merck TLC silica gel 60 F254 or Macherey-Nagel POLYGRAM ALOX N/UV254.

^1H NMR chemical shifts are reported in parts per million (ppm) relative to the solvent residual peak ($CDCl_3$, 7.26 ppm). Multiplicities are given as: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), m (multiplet), and the coupling constants, *J*, are given in Hz. ^{13}C NMR chemical shifts are reported relative to the solvent residual peak ($CDCl_3$, 77.0 ppm).

Scheme 2. Synthesis of Phenylthioureas 1−3



IR frequencies are given in cm^{-1}. HRMS data were acquired on Bruker maXis UHPLC-HR-MS QTOF instrument with an ESI source. All solid synthetic products were noncrystalline (oils or sticky solids), precluding melting point determination.

Cyclic voltammograms conditions: 1 mM compound, 0.1 M Bu_4NClO_4 as supporting electrolyte in CH_3CN, scan rate 100 mV s^{-1}, glassy carbon working electrode (Ø = 0.3 cm), Pt wire counter electrode, Ag/AgCl reference electrode, added ferrocene (Fc) as internal reference.

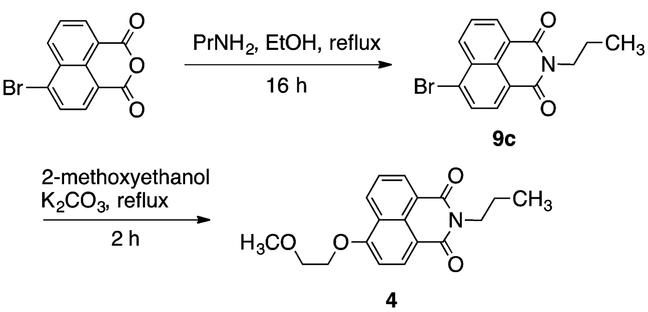
Fluorescence measurements were carried out in spectroscopic grade CH_3OH using a 450 W xenon lamp excitation with 1 nm excitation and 1 nm emission slit widths. Emission spectra were obtained by exciting at the longest-wavelength absorption maxima. Quantum yields were determined by standard methods[24] using anthracene (ϕ = 0.30) in CH_3OH.[25] The samples were diluted to optical transparency ($A \leq 0.05$), and the integrated emission intensity was compared to an iso-absorptive solution of the standards in degassed solvent.

For extinction coefficient determination, four independent solutions of different concentration were prepared, with absorption between 0.04 and 0.10 AU. The value of ε was calculated by linear least-squares fitting of plots of A vs concentration. All fits gave R^2 values of ≥0.98.

General Protocol for Metal Ion Titrations. Chemosensor solutions (3.00 mL, ca. 3.3 μM in CH_3OH, prepared by serial dilution) were placed in a quartz cuvette, and the initial fluorescence emission spectra were recorded. Aliquots of metal salt (3 × 5 μL, 20 μM−2 M in 10-fold increments, in CH_3OH) were then added sequentially (i.e., 3 × 5 μL × 20 μM, then 3 × 5 μL × 20 μM, etc.) until maximum fluorescence increase or a total volume of 4 mL was reached. After each addition of the metal ion solutions, the fluorescence emission spectra were recorded.

Synthetic Schemes. Scheme 2−4 show the synthetic schemes for each synthesis.

Scheme 3. Synthesis of Reference Compound 4



General Procedures. The following are general protocols, illustrated with the transformation of **9**→**13**. Complete experimental procedures follow.

(A) *N*-Boc-protected amine **9** (1.0 equiv) was dissolved in DMF and triethylamine (1.2 equiv) was added. The reaction mixture was stirred at room temperature for 30 min. 4-Bromo-1,8-naphthalic anhydride (1.0 equiv) was added as a solid and the reaction mixture was heated to 100 °C for 48 h. The solvent was removed and the crude product was purified by column chromatography to give **10**.

(B) A solution of **10** (1 equiv) and potassium carbonate (10 equiv) in methoxyethanol was heated to 100 °C for 2 h. After the solution was cooled to room temperature, water was added and the product was extracted with CH_2Cl_2. The organic phase was washed with water, dried over $MgSO_4$, and concentrated under vacuum. The crude product was purified by column chromatography to give **11**.

(C) A solution of **11** (1 equiv) in CH_2Cl_2 was cooled to 0 °C, and trifluoroacetic acid (25 equiv) was added. The reaction mixture was stirred for 2 h at room temperature. The reaction was quenched by the adding of a solution of NaOH (2 N), and the product was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$ and concentrated under vacuum. The product, **12**, did not require further purification.

(D) Phenyl isothiocyanate (1.0 equiv) was added to a suspension of **12** (1 equiv) in acetonitrile. The reaction mixture was stirred at room temperature for 30 min. The resulting solid was isolated by filteration and purified by recrystallization from 2-propanol to give **1**.

(E) 1,1-Thiocarbonyldiimidazole and **12** were mixed in acetonitrile, and the reaction mixture was stirred for 1 h at room temperature. The solid product **13** was obtained by filtration and did not require purification.

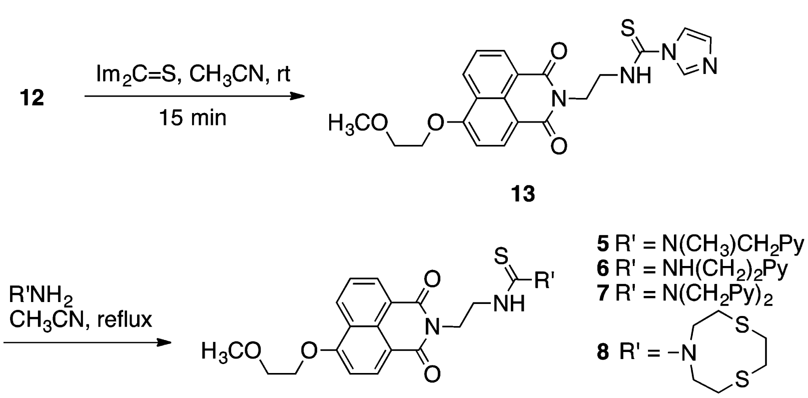
(F) To a mixture of **13** (1 equiv) and acetonitrile was added a solution of the appropriate amine (1.0 equiv) in acetonitrile. The reaction mixture was refluxed until clarified. After cooling to room temperature, the reaction mixture was concentrated under vacuum. The residue was solved in CH_2Cl_2 (20 mL), washed with water, dried over $MgSO_4$, and concentrated under vacuum. The crude was purified to give **5**−**8** as yellow solids.

Synthetic Details and Tabulated Spectroscopic Data. *N-Boc-diamine 9.* Prepared according to the literature.[26] The ^{1}H NMR data are consistent with those previously reported. ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 4.92 (s, br, 1H), 3.15 (q, 2H, J = 5.8), 2.78 (t, 2H, J = 5.8), 1.43 (s, 9H), 1.19 (s, br, 2H).

N-Boc-bromonaphthalimide Amine 10. Prepared according to general procedure A: *N*-Boc-1,2-diaminoethane **9** (400 mg, 2.71 mmol) in DMF (20 mL); triethylamine (328 mg, 3.25 mmol); 4-bromo-1,8-naphthalic anhydride (750 mg, 2.71 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 99.5/0.5) to give **10** as a yellow solid (782 mg, 69%). The ^{1}H NMR data are consistent with those previously reported.[27] ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.66 (dd, 1H, J = 7.3, J = 0.9), 8.57 (dd, 1H, J = 8.4, J = 0.8), 8.42 (d, 1H, J = 7.8), 8.04 (d, 1H, J = 7.8), 7.86 (dd, 1H, J = 8.4, J = 7.3), 4.93 (s, br, 1H), 4.35 (t, 2H, J = 5.7), 3.55−3.52 (m, 2H), 1.28 (s, 9H).

N-Boc-methoxyethoxynaphthalimide Amine 11. Prepared according to general procedure B: **10** (800 mg, 1.90 mmol); potassium

Scheme 4. Synthesis of Chemosensors 5−8 (Py = 2-Pyridyl)



dx.doi.org/10.1021/jo4003129 | J. Org. Chem. XXXX, XXX, XXX−XXX

carbonate (2.63 g, 19.00 mmol); methoxyethanol (50 mL). The crude product was purified by column chromatography (CH_2Cl_2/EtOAc 9/1) to give **11** as a light yellow solid (665 mg, 84%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.61 (dd, 1H, J = 1.2, J = 8.3), 8.60 (dd, 1H, J = 1.2, J = 7.4), 8.54 (d, 1H, J = 8.3), 7.70 (dd, 1H, J = 7.4, J = 8.3), 7.04 (d, 1H, J = 8.3), 5.02 (s, 1H, br), 4.44−4.42 (m, 2H), 4.35 (t, 2H, J = 5.7), 3.95−3.93 (m, 2H), 3.54−3.49 (m, 5H), 1.31 (s, 9H). ^{13}C NMR (δ ppm, 100 MHz, $CDCl_3$): 165.0, 164.4, 160.3, 156.1, 133.7, 132.0, 129.7, 129.1, 126.1, 123.7, 122.3, 115.2, 106.1, 79.2, 70.8, 68.6, 59.5, 40.1, 39.8, 28.4 (3). IR (KBr) cm^{-1}: 3346m, 2979m, 2932m, 2889m, 1698s, 1655s, 1622m, 1595s, 1582s, 1535s, 1453m, 1428m, 1390s, 1362s, 1270s, 1237s, 1180s, 1129m, 1087m, 1054m. HRMS-ESI: calcd for $C_{22}H_{26}N_2NaO_6$ [M + Na]$^+$ 437.16831, found 437.16787. R_f (CH_2Cl_2/AcOEt 9/1): 0.14.

*Methoxyethoxynaphthalimide Amine **12**.* Prepared according to general procedure C: **11** (640 mg, 1.54 mmol) in CH_2Cl_2 (5 mL); trifluoroacetic acid (4.40 g, 38.60 mmol). Compound **12** was obtained without purification as a yellow solid (393 mg, 81%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.62 (dd, 1H, J = 1.2, J = 8.3), 8.60 (dd, 1H, J = 1.2, J = 7.4), 8.54 (d, 1H, J = 8.3), 7.70 (dd, 1H, J = 7.4, J = 8.3), 7.04 (d, 1H, J = 8.3), 4.44−4.42 (m, 2H), 4.28 (t, 2H, J = 6.5), 3.95−3.93 (m, 2H), 3.52 (s, 3H), 3.08 (t, 2H, J = 6.5). ^{13}C NMR (δ ppm, 100 MHz, $CDCl_3$): 165.0, 164.4, 160.3, 133.7, 131.9, 129.6, 129.1, 126.1, 123.7, 122.4, 115.3, 106.1, 70.8, 68.6, 59.5, 43.1, 40.8. IR (KBr) cm^{-1}: 3366m, 3299w, 2953w, 2878w, 2821w, 1696s, 1656s, 1622m, 1514s, 1473m, 1457m, 1427m, 1386s, 1356s, 1310m, 1263s, 1233s, 1200m, 1172m, 1124s, 1106s, 1093s, 1075s, 1033s. HRMS-ESI: calcd for $C_{17}H_{19}N_2O_4$ [M + H]$^+$ 315.13393, found 315.13342.

*Phenylthiourea Naphthalimide **1**.* Prepared according to general procedure D: Phenyl isothiocyanate (86 mg, 0.64 mmol); **12** (200 mg, 0.64 mmol); acetonitrile (8 mL). The reaction mixture was stirred at room temperature for 30 min. The solvent was removed, and the crude material was purified by recrystallization from 2-propanol to give **1** as a cream white solid (185 mg, 65%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.65 (dd, 1H, J = 1.2, J = 8.3), 8.54 (dd, 1H, J = 1.2, J = 7.4), 8.48 (d, 1H, J = 8.3), 7.72 (dd, 1H, J = 7.4, J = 8.3), 7.43−7.34 (m, 3H), 7,20 (s, br), 7.07 (d, 1H, J = 8.3), 6.85 (s, 1H, br), 4.46−4.41 (m, 4H), 4.01−3.94 (m, 4H), 3.53 (s, 3H). ^{13}C NMR (δ ppm, 125 MHz, $CDCl_3$): 181.4, 165.1, 164.5, 160.6, 136.1, 134.0, 132.1, 130.1 (2), 129.6, 129.5, 127.5, 126.1, 126.0 (2), 123.7, 122.0, 114.8, 106.2, 70.8, 68.6, 59.6, 46.0, 38.7. IR (KBr) cm^{-1}: 3361m, 3193m, 3003w, 2935w, 2885w, 1690s, 1651s, 1620m, 1595s, 1549s, 1515s, 1471m, 1453m, 1427m, 1398m, 1385s, 1362s, 1345s, 1321s, 1298m, 1271s, 1238s, 1202m, 1174m, 1124s, 1081s, 1033m. HRMS-ESI: calcd for $C_{24}H_{23}N_3NaO_4S$ [M + Na]$^+$ 472.13015, found 437.12983. R_f (deactivated silica gel, CH_2Cl_2/hexane/Et_3N 3.5/1.5/0.1): 0.42.

*N-Boc-diamine **9a**.* Prepared according to the literature.[28] The ^{1}H NMR data are consistent with those previously reported. ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 4.91 (s, br, 1H), 3.23−3.15 (m, 2H), 2.75 (t, 2H, J = 6.6), 1.60 (quint, 2H, J = 6.6), 1.43 (s, 9H), 1.29 (s, br, 2H).

*N-Boc-bromonaphthalimide Amine **10a**.* Prepared according to general procedure A: N-Boc-1,3-diaminopropane **9a** (1.00 g, 5.74 mmol) was dissolved in DMF (20 mL); triethylamine (0.58 g, 5.74 mmol); 4-bromo-1,4-naphthalic anhydride (1.59 g, 5.74 mmol) The crude product was purified by column chromatography (CH_2Cl_2/MeOH 99.5/0.5) to give **10a** as a yellow solid (2.38 g, 94%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.66 (dd, 1H, J = 7.3, J = 1.2), 8.58 (dd, 1H, J = 8.5, J = 1.2), 8.41 (d, 1H, J = 7.8), 8.05 (d, 1H, J = 7.8), 7.85 (dd, 1H, J = 8.5, J = 7.3), 5.20 (s, br, 1H), 4.26 (t, 2H, J = 6.6), 3.19−3.14 (m, 2H), 1.93 (quint, 2H, J = 6.5), 1.45 (s, 9H). ^{13}C NMR (δ ppm, 125 MHz, $CDCl_3$): 164.0 (2), 156.0, 133.5, 132.3, 131.4, 131.2, 130.7, 130.5, 129.0, 128.1, 122.9, 122.0, 79.1, 37.8 (2), 37.5, 28.4 (3). IR (KBr) cm^{-1}: 3359m, 2968m, 2926w, 1704s, 1684s, 1652s, 1618m, 1592m, 1571m, 1526s, 1460m, 1447m, 1436m, 1361s, 1349s, 1283s, 1269s, 1251m, 1230s, 1171s, 1101m, 1080m, 1065m, 1044m, 1001w. HRMS-ESI: calcd for $C_{20}H_{21}BrN_2NaO_4$ [M + Na]$^+$ 455.05769, found 455.05746. R_f (CH_2Cl_2/MeOH 99.5/0.5): 0.23.

*N-Boc-methoxyethoxynaphthalimide Amine **11a**.* Prepared according to general procedure B: **10a** (1.00 g, 2.30 mmol); potassium carbonate (3.18 g, 23.00 mmol); methoxyethanol (60 mL). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 99/1) to give **11a** as a light yellow solid (720 mg, 73%). ^{1}H NMR (δ ppm, 500 MHz, $CDCl_3$): 8.63 (dd, 1H, J = 1.2, J = 8.3), 8.61 (dd, 1H, J = 1.2, J = 7.4), 8.55 (d, 1H, J = 8.3), 7.72 (dd, 1H, J = 7.4, J = 8.3), 7.06 (d, 1H, J = 8.3), 5.31 (s, 1H, br), 4.45−4.43 (m, 2H), 4.26 (t, 2H, J = 6.5), 3.95−3.93 (m, 2H), 3.52 (s, 3H), 3.18−3.14 (m, 2H), 1.93 (quint, 2H, J = 6.4), 1.45 (s, 9H). ^{13}C NMR (δ ppm, 100 MHz, $CDCl_3$): 165.0, 164.4, 160.3, 156.2, 133.7, 131.9, 129.6, 129.2, 126.1, 123.7, 122.3, 115.2, 106.2, 79.1, 70.8, 68.6, 59.5, 37.6 (3), 28.6 (3). IR (KBr) cm^{-1}: 3309m, 2930m, 2895w, 2819w, 1688s, 1658s, 1623w, 1598m, 1538m, 1442m, 1398m, 1363s, 1324w, 1290m, 1267m, 1244s, 1180m, 1165m, 1127s, 1097m, 1084m, 1060w, 1036w. HRMS-ESI: calcd for $C_{23}H_{28}N_2NaO_6$ [M + Na]$^+$ 451.18369, found 451.18363. R_f (CH_2Cl_2/EtOAc 9/1): 0.16.

*Methoxyethoxynaphthalimide Amine **12a**.* Prepared according to general procedure C: **11a** (350 mg, 0.82 mmol) in CH_2Cl_2 (8 mL); trifluoroacetic acid (2.33 g, 38.6 mmol). Compound **12a** was obtained without as a yellow solid without need for further purification (220 mg, 81%). ^{1}H NMR (δ ppm, 500 MHz, $CDCl_3$): 8.63 (dd, 1H, J = 1.2, J = 8.3), 8.61 (dd, 1H, J = 1.2, J = 7.4), 8.55 (d, 1H, J = 8.3), 7.72 (dd, 1H, J = 7.4, J = 8.3), 7.06 (d, 1H, J = 8.3), 4.45−4.42 (m, 2H), 4.28 (t, 2H, J = 6.8), 3.95−3.93 (m, 2H), 3.53 (s, 3H), 2.76 (t, 2H, J = 6.7). ^{13}C NMR (δ ppm, 125 MHz, $CDCl_3$): 164.9, 164.3, 160.2, 133.6, 131.9, 129.6, 129.1, 126.1, 123.7, 122.4, 115.3, 106.1, 70.8, 68.5, 59.6, 39.5, 37.7, 32.3. IR (KBr) cm^{-1}: 3372w, 3308w, 2955w, 2934w, 2896w, 2866,w 2822w, 1695s, 1658s, 1621m, 1594s, 1579m, 1515w, 1472w, 1454w, 1431m, 1389s, 1354s, 1271s, 1240m, 1199m, 1129m, 1094m, 1044m, 1034m. HRMS-ESI: calcd for $C_{18}H_{20}N_2O_4$ [M + H]$^+$ 329.14958, found 329.14967.

*Naphthalimide Phenylthiourea **2**.* Prepared according to general procedure D: Phenyl isothiocyanate (37 mg, 0.27 mmol); **12a** (90 mg, 0.27 mmol); acetonitrile (6 mL). The reaction mixture was stirred at room temperature for 30 min, the volatiles were removed, and the crude product was purified by recrystallization from 2-propanol to give **2** as a yellow solid (75 mg, 59%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.63 (d, 1H, J = 8.3), 8.52 (d, 1H, J = 7.4), 8.46 (d, 1H, J = 8.3), 7.70 (dd, 1H, J = 7.4, J = 8.3), 7.56 (s, 1H, br), 7.52−7.48 (m, 2H), 7.35−7.30 (m, 3H), 7.05 (d, 1H, J = 8.3), 4.44−4.42 (m, 2H), 4.16 (t, 2H, J = 6.1), 3.95−3.93 (m, 2H), 3.69−3.67 (m, 2H), 3.52 (s, 3H), 2.09−2.06 (m, 2H). ^{13}C NMR (δ ppm, 125 MHz, $CDCl_3$): 180.64, 165.05, 164.41, 160.42, 136.29, 133.87, 132.03, 130.21 (2), 129.52, 129.45, 127.19, 126.10, 125.32 (2), 123.64, 122.08, 114.88, 106.14, 70.75, 68.57, 59.54, 42.30, 37.36, 27.45. IR (KBr) cm^{-1}: 3308m, 3198m, 3104w, 3025w, 2932w, 1689s, 1653s, 1591s, 1548s, 1532s, 1513s, 1457m, 1395s, 1376m, 1357s, 1343s, 1327s, 1315s, 1266s, 1237s, 1171s, 1123m, 1081s, 1051m. HRMS-ESI: calcd for $C_{25}H_{25}N_3NaO_4S$ [M + Na]$^+$ 486.1458, found 486.14522. R_f (deactivated silica gel, CH_2Cl_2/hexane/Et_3N 3.5/1.5/0.1): 0.44.

*N-Boc-diamine **9b**.* Prepared according to the literature.[29] The ^{1}H NMR data are consistent with those previously reported. ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 4.61 (s, br, 1H), 3.15−3.11 (m, 2H), 2.71 (t, 2H, J = 6.6), 1.50−1.46 (m, 4H), 1.44 (s, 9H), 1.31 (s, br, 2H).

*N-Boc-bromonaphthalimide Amine **10b**.* Prepared according to general procedure A: N-Boc-1,4-diaminobutane **9b** (1.30 g, 6.91 mmol) was dissolved in DMF (20 mL); triethylamine (0.70 g, 6.91 mmol); 4-bromo-1,4-naphthalic anhydride (1.91 g, 6.90 mmol). The crude product was purified by column chromatography (CH_2Cl_2/MeOH 99.5/0.5) to give **10b** as a light yellow solid (2.10 g, 68%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.65 (dd, 1H, J = 7.3, J = 1.1), 8.57 (dd, 1H, J = 8.5, J = 1.1), 8.41 (d, 1H, J = 7.8), 8.04 (d, 1H, J = 7.8), 7.85 (dd, 1H, J = 8.5, J = 7.3), 4.61 (s, br, 1H), 4.19 (t, 2H, J = 7.4), 3.20 (q, 2H, J = 6.5), 1.80−1.73 (m, 2H), 1.65−1.57 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (δ ppm, 125 MHz, $CDCl_3$): 163.8 (2), 156.1, 133.5, 132.2, 131.4, 131.3, 130.8, 130.5, 129.2, 128.2, 123.2, 122.4, 79.2, 40.4, 40.2, 28.6 (3), 27.7, 25.55. IR (KBr) cm^{-1}: 3358m, 2971w, 2934w, 1703s, 1682s, 1650s, 1591m, 1570m, 1532m, 1460w, 1435w, 1389m, 1364m, 1263m, 1233m, 1177m, 1104w, 1066m. HRMS-ESI: calcd for $C_{21}H_{23}BrN_2NaO_4$ [M + Na]$^+$ 469.07334, found 469.07344. R_f (CH_2Cl_2/MeOH 99.5/0.5): 0.23.

*N-Boc-methoxyethoxynaphthalimide Amine **11b**.* Prepared according to general procedure B: **10b** (1.75 g, 3.91 mmol); potassium carbonate (5.4 g, 39.1 mmol); methoxyethanol (60 mL). The crude product was purified by column chromatography (CH_2Cl_2/AcOEt 9/1) to give **11b** as a light yellow solid (1.37 g, 79%). ^{1}H NMR (δ ppm, 500 MHz, $CDCl_3$): 8.62 (dd, 1H, J = 1.2, J = 8.3), 8.61 (dd, 1H, J = 1.2, J = 7.4), 8.54 (d, 1H, J = 8.3), 7.71 (dd, 1H, J = 7.4, J = 8.3), 7.06 (d, 1H, J = 8.3), 4.63 (s, 1H, br), 4.45−4.43 (m, 2H), 4.18 (t, 2H, J = 7.4), 3.95−3.93 (m, 2H), 3.52 (s, 3H), 3.20−3.19 (m, 2H), 1.80−1.74 (m, 2H), 1.64−1.58 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (δ ppm, 100 MHz, $CDCl_3$): 164.7, 164.1, 160.2, 156.1, 133.5, 131.8, 129.6, 129.0, 126.1, 123.7, 122.5, 115.4, 106.1, 79.1, 70.8, 68.5, 59.5, 40.4, 39.9, 28.6 (3), 27.7, 25.6. IR (KBr) cm^{-1}: 3347m, 2980m, 2936m, 2893w, 1683s, 1652s, 1623m, 1596m, 1541s, 1453m, 1393s, 1358s, 1292m, 1274s, 1248s, 1175s, 1125m, 1097m, 1085m, 1052m, 1032m. HRMS-ESI: calcd for $C_{24}H_{30}N_2NaO_6$ [M + Na]$^+$ 465.19961, found 465.19935. R_f (CH_2Cl_2/AcOEt 9/1): 0.16.

*Methoxyethoxynaphthalimide Amine **12b**.* Prepared according to general procedure C: **11b** (560 mg, 1.26 mmol) in CH_2Cl_2 (10 mL); trifluoroacetic acid (3.6 g, 31.6 mmol). Compound **12b** was obtained without purification as a yellow solid (560 mg, 84%). ^{1}H NMR (δ ppm, 500 MHz, $CDCl_3$): 8.57 (dd, 1H, J = 1.2, J = 8.3), 8.55 (dd, 1H, J = 1.2, J = 7.4), 8.49 (d, 1H, J = 8.3), 7.67 (dd, 1H, J = 7.4, J = 8.3), 7.02 (d, 1H, J = 8.3), 4.42−4.40 (m, 2H), 4.17 (t, 2H, J = 7.3), 3.94−3.92 (m, 2H), 3.52 (s, 3H), 2.91 (t, 2H, J = 7.0), 1.85−1.79 (m, 2H), 1.72−1.66 (m, 2H). ^{13}C NMR (δ ppm, 100 MHz, $CDCl_3$): 164.7, 164.1, 160.2, 133.5, 131.8, 129.6, 129.0, 126.1, 123.7, 122.5, 115.4, 106.1, 70.8, 68.5, 59.5, 41.9, 40.0, 31.1, 25.5. IR (KBr) cm^{-1}: 3340w, 2928w, 1694s, 1657s, 1622m, 1594s, 1514m, 1454m, 1429m, 1389s, 1354s, 1271s, 1240m, 1200m, 1181m, 1129m, 1094m, 1080m, 1032m, 1053m. HRMS-ESI: calcd for $C_{19}H_{22}N_2O_4$ [M + H]$^+$ 343.16523, found 343.6529.

*Naphthalimide Phenylthiourea **3**.* Prepared according to general procedure D: Phenyl isothiocyanate (100 mg, 0.73 mmol); **12b** (252 mg, 0.73 mmol); acetonitrile (10 mL). The reaction mixture was stirred at room temperature for 30 min, the solvent evaporated and the crude product was purified by recrystallization from 2-propanol to give **3** as a cream white solid (175 mg, 50%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.63 (dd, 1H, J = 1.2, J = 8.3), 8.56 (dd, 1H, J = 1.2, J = 7.4), 8.49 (d, 1H, J = 8.3), 7.71 (dd, 1H, J = 7.4, J = 8.3), 7.56 (s, 1H, br), 7.44−7.40 (m, 2H), 7.31−7.22 (m, 3H), 7.05 (d, 1H, J = 8.3), 6.26 (s, 1H, br), 4.45−4.43 (m, 2H), 4.17 (t, 2H, J = 7.0), 3.95−3.93 (m, 2H), 3.78−3.73 (m, 2H), 3.52 (s, 3H), 1.81−1.68 (m, 4H). ^{13}C NMR (δ ppm, 125 MHz, $CDCl_3$): 180.9, 164.7, 164.1, 160.3, 136.3, 133.7, 131.9, 130.3 (2), 129.6, 129.2, 127.4, 126.1, 125.6 (2), 123.7, 122.4, 115.2, 106.1, 70.8, 68.6, 59.6, 45.1, 39.5, 26.4, 25.5. IR (KBr) cm^{-1}: 3379m, 3159m, 2933m, 1692s, 1650s, 1595s, 1578s, 1541s, 1513s, 1450m, 1397s, 1386s, 1358s, 1320s, 1266s, 1235s, 1189m, 1126s, 1087s, 1060m, 1029m. HRMS-ESI: calcd for $C_{26}H_{27}N_3NaO_4S$ [M + Na]$^+$ 500.16145, found 500.16158. R_f (deactivated silica gel, CH_2Cl_2/hexane/Et_3N 3.5/1.5/0.1): 0.36.

*N-Propylbromonaphthalimide **9c**.* 1-Aminopropane (43 mg, 0.72 mmol) was added to 4-bromo-1,8-naphthalic anhydride (200 mg, 0.72 mmol) in ethanol (15 mL), resulting in an orange solution which was refluxed overnight. After the mixture was cooled to room temperature a solid precipitated and was isolated by filtration. Purification by column chromatography (CH_2Cl_2/hexane 1/1) to give **9c** as a white solid (200 mg, 88%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.67 (dd, 1H, J = 1.1, J = 7.3), 8.58 (dd, 1H, J = 1.1, J = 8.5), 8.42 (d, 1H, J = 7.9), 8.05 (d, 1H, J = 7.9), 7.85 (dd, 1H, J = 7.3, J = 8.5), 4.17−4.13 (m, 2H), 1.82−1.73 (m, 2H), 1.02 (t, 3H, J = 7.4). ^{13}C NMR (δ ppm, 125 MHz, $CDCl_3$): 163.7 (2), 133.3, 132.1, 131.3, 131.2, 130.8, 130.3, 129.2, 128.2, 123.3, 122.4, 42.2, 21.5, 11.6. IR (KBr) cm^{-1}: 3054w, 2950m, 2869w, 1699s, 1659s, 1586s, 1568s, 1503m, 1460m, 1437m, 1397m, 1358s, 1287m, 1240s, 1165m, 1152w, 1101w, 1072s, 1044m, 1015w. HRMS-ESI: calcd for $C_{15}H_{12}BrNNaO_2$ [M + Na]$^+$ 339.99436, found 339.99412. R_f (CH_2Cl_2/hexane 1/1): 0.42.

*N-Propylmethoxyethoxynaphthalimide **4**.* Prepared according to general procedure B: **9c** (100 mg, 0.32 mmol); potassium carbonate (435 mg, 19.00 mmol); methoxyethanol (10 mL). The crude product was purified by column chromatography (CH_2Cl_2 to CH_2Cl_2/MeOH 99/1) to give **4** as a light yellow solid (71 mg, 72%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.67−8.59 (m, 2H), 8.55 (d, 1H, J = 8.2), 7.71 (t, 1H, J = 7.8), 7.05 (d, 1H, J = 8.3), 4.51−4.36 (m, 2H), 4.17−4.18 (m, 2H), 4.04−3.87 (m, 2H), 3.52 (s, 3H), 1.88−1.66 (m, 2H), 1.01 (t, 3H, J = 7.4). ^{13}C NMR (δ ppm, 125 MHz, $CDCl_3$): 164.7, 164.2, 160.1, 133.5, 131.8, 129.6, 128.9, 126.1, 123.7, 122.7, 115.5, 106.1, 70.8, 68.5, 59.6, 42.0, 21.6, 11.7. IR (KBr) cm^{-1}: 2960w, 2931w, 2900w, 2875w, 1699s, 1660s, 1595s, 1580m, 1514w, 1455m, 1432m, 1387s, 1354s, 1287s, 1236s, 1200m, 1130m, 1091s, 1045m, 1037m. HRMS-ESI: calcd for $C_{18}H_{19}NNaO_4$ [M + Na]$^+$ 336.12063, found 336.12065. R_f (CH_2Cl_2/MeOH 99/1): 0.28.

*Naphthalimide Imidazolylthiourea **13**.* Prepared according to general procedure E: thiocarbonyldiimidazole (187 mg, 1.05 mmol), **12** (330 mg, 1.05 mmol), acetonitrile (5 mL). **13** was obtained as a yellow solid without need for purification (365 g, 82%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 9.13 (s, 1H), 8.62 (dd, 1H, J = 1.2, J = 8.3), 8.60 (dd, 1H, J = 1.2, J = 7.4), 8.54 (d, 1H, J = 8.3), 8.42 (s, 1H), 7.70 (dd, 1H, J = 7.4, J = 8.3), 7.64 (s, 1H), 7.04 (d, 1H, J = 8.3), 4.63−4.60 (m, 2H), 4.47−4.44 (m, 2H), 4.05−4.04 (m, 2H), 3.96−3.93 (m, 2H), 3.52 (s, 3H). ^{13}C NMR (δ ppm, 100 MHz, $CDCl_3$): 166.0, 161.1, 136.7, 134.6, 132.6, 130.1, 129.7, 126.4, 123.7, 121.7, 117.0, 114.4, 106.5, 70.7, 68.8, 59.6, 47.8, 38.8. HRMS-ESI: calcd for $C_{21}H_{20}N_4O_4S$ [M + H]$^+$ 425.12780, found 425.12724.

*N-Methyl-2-picolylthiourea Naphthalimide **5**.* Prepared according to general procedure F: **13** (150 mg, 0.35 mmol); acetonitrile (10 mL); *N*-methyl-2-picoline (43 mg, 0.35 mmol); acetonitrile (3 mL). The reaction mixture was stirred for 1 h at reflux, the volatiles were removed, and the crude product was purified by column chromatography (silica gel deactivated with 20% Et_3N/hexanes, CH_2Cl_2/hexane/Et_3N 3.5/1.5/0.1) to give **5** as a yellow solid (138 mg, 82%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.65 (dd, 1H, J = 1.2, J = 8.4), 8.58 (dd, 1H, J = 1.2, J = 7.3), 8.53 (d, 1H, J = 8.3), 8.44−8.43 (m, 1H), 7.72 (dd, 1H, J = 7.3, J = 8.4), 7.58 (td, J = 1.6, J = 7.6, 1H), 7.37−7.32 (m, 2H), 7.14−7.11 (m, 1H), 7.06 (d, 1H, J = 8.4), 5.13 (s, 2H), 4.55−4.52 (m, 2H), 4.46−4.43 (m, 2H), 4.04−4.01 (m, 2H), 3.96−3.94 (m, 2H), 3.53 (s, 3H), 3.21 (s, 3H). ^{13}C NMR (δ ppm, 100 MHz, $CDCl_3$): 182.9, 165.6, 165.0, 160.6 (2), 149.3, 137.0, 134.0, 132.2, 129.7, 129.5, 126.2, 123.7, 122.5, 122.3, 122.1, 114.9, 106.3, 70.8, 68.7, 59.6, 58.6, 47.4 (2), 39.4. IR (KBr) cm^{-1}: 3343w, 2925w, 1693s, 1652s, 1621m, 1593s, 1534s, 1472m, 1436m, 1386s, 1354s, 1269s, 1236s, 1179m, 1125m, 1081s, 1031m. HRMS-ESI: calcd for $C_{25}H_{26}N_4O_4S$ [M + H]$^+$ 479.17475, found 479.17452. R_f (deactivated silica gel, CH_2Cl_2/hexane/Et_3N 3.5/1.5/0.1): 0.26.

*Ethylpyridylthiourea Naphthalimide **6**.* Prepared according to general procedure F: **13** (150 mg, 0.35 mmol); acetonitrile (10 mL); 2-(aminoethyl)pyridine (43 mg, 0.35 mmol); acetonitrile (3 mL). The reaction mixture was stirred for 1 h at reflux, the solvent was removed under vacuum, and the solid crude product was washed with acetonitrile and purified by recrystallization from 2-propanol to give **6** as a yellow solid (96 mg, 57%). ^{1}H NMR (δ ppm, 500 MHz, $CDCl_3$): 8.64 (dd, 1H, J = 1.2, J = 8.4), 8.60 (dd, 1H, J = 1.2, J = 7.3), 8.55 (d, 1H, J = 8.3), 8.54−8.52 (m, 1H), 7.72 (dd, 1H, J = 7.3, J = 8.4), 7.63 (td, J = 1.6, J = 7.6, 1H), 7.24−7.22 (m, 2H), 7.16−7.13 (m, 1H), 7.06 (d, 1H, J = 8.4), 5.13, 4.46−4.42 (m, 4H), 3.96−3.93 (m, 4H), 3.80−3.70 (m, 2H), 3.53 (s, 3H), 3.15−3.12 (m, 2H). ^{13}C NMR (δ ppm, 100 MHz, $CDCl_3$): 181.7, 165.3, 164.7, 160.4 (2), 149.3, 136.8, 134.1, 132.2, 129.6, 126.2, 123.7, 123.6, 122.0, 121.8, 114.7, 106.3, 70.8, 68.6, 59.6, 53.6, 38.8, 36.7, 25.5. IR (KBr) cm^{-1}: 3350 (m), 3210w, 2928w, 1692s, 1657s, 1593s, 1581s, 1518s, 1474m, 1425m, 1386s, 1346s, 1272s, 1293s, 1196m, 1124m, 1083s, 1054m. HRMS-ESI: calcd for $C_{25}H_{26}N_4O_4S$ [M + H]$^+$ 479.17475, found 479.17497. R_f (deactivated silica gel, CH_2Cl_2/hexane/Et_3N 3.5/1.5/0.1): 0.09.

*Dipicolylthiourea Naphthalimide **7**.* Prepared according to general procedure F: **13** (150 mg, 0.35 mmol); acetonitrile (10 mL); di(2-picolyl)amine (70 mg, 0.35 mmol); acetonitrile (3 mL). The reaction mixture was stirred for 1 h at reflux, the solvent was removed, and the crude product was purified by column chromatography (silica gel deactivated with 20% Et_3N/hexanes, CH_2Cl_2/hexane/Et_3N 3.5/1.5/

0.1) to give **7** as a yellow solid (180 mg, 82%). ^{1}H NMR (δ ppm, 500 MHz, $CDCl_3$): 8.77 (t, 1H, J = 4.8), 8.63 (dd, 1H, J = 1.2, J = 8.4), 8.52 (dd, 1H, J = 1.2, J = 7.3), 8.48 (d, 1H, J = 8.3), 8.32−8.28 (m, 2H), 7.68 (dd, 1H, J = 7.3, J = 8.4), 7.48 (td, J = 1.8, J = 7.6, 2H), 7.26−7.23 (m, 2H), 7.06−7.02 (m, 3H), 4.97 (s, 4H), 4.55−4.53 (m, 2H), 4.45−4.42 (m, 2H), 4.16−4.12 (m, 2H), 3.96−3.93 (m, 2H), 3.53 (s, 3H). ^{13}C NMR (δ ppm, 100 MHz, $CDCl_3$): 184.9, 165.1, 164.4, 160.3 (3), 149.0 (2), 136.9 (2), 133.7, 131.9, 129.7, 129.1, 126.1, 123.7, 122.9 (2), 122.5 (2), 122.4, 115.3, 106.2, 70.8, 68.6, 59.5, 45.9, 45.6 (2), 39.6. IR (KBr) cm^{-1}: 3374w, 2924m, 1695s, 1656s, 1592s, 1516m, 1473m, 1440m, 1384s, 1355s, 1267s, 1325s, 1201m, 1179m, 1125m, 1081s, 1031m. HRMS-ESI: calcd for $C_{30}H_{29}N_5O_4S$ $[M + H]^+$ 556.2013, found 556.20181. R_f (deactivated silica gel, CH_2Cl_2/Hexane/Et_3N 3.5/1.5/0.1): 0.26.

*Thiacrown Amine **8a**.* Prepared according to the literature.[30] The ^{1}H NMR data are consistent with those previously reported.[31] ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 3.02−2.99 (m, 8H), 2.82−2.79 (m, 4H).

*Thiacrown Thiourea Naphthalimide **8**.* Prepared according to general procedure F: **13** (50 mg, 0.12 mmol); acetonitrile (3 mL); thiacrown amine **8a** (19 mg, 0.12 mmol); acetonitrile (3 mL). The reaction mixture was stirred for 30 min at reflux. The solvent was removed under vacuum and the solid crude product was washed with acetonitrile and purified by recrystallization from 2-propanol to give **8** as a yellow solid (42 mg, 69%). ^{1}H NMR (δ ppm, 400 MHz, $CDCl_3$): 8.66 (dd, 1H, J = 1.2, J = 8.4), 8.62 (dd, 1H, J = 1.2, J = 7.4), 8.56 (d, 1H, J = 8.3), 7.73 (dd, 1H, J = 7.4, J = 8.4), 7.33 (s, 1H), 7.07 (d, 1H, J = 8.4), 4.55−4.53 (m, 2H), 4.45−4.43 (m, 2H), 4.02−3.94 (m, 8H), 3.53 (s, 3H), 3.31−3.30 (m, 2H), 2.80 (s, 4H). ^{13}C NMR (δ ppm, 100 MHz, $CDCl_3$): 184.0, 165.4, 164.8, 160.5, 133.9, 132.0, 129.5, 129.4, 126.1, 123.6, 122.0, 114.8, 106.1, 70.6, 68.5, 59.4, 56.5 (2), 46.8, 39.2, 34.0, 30.7 (2), 25.4. IR (KBr) cm^{-1}: 3399m, 2921w, 1691s, 1647s, 1619m, 1594s, 1580s, 1525s, 1470m, 1441m, 1421m, 1384s, 1345s, 1330s, 1265s, 1238s, 1198m, 1166m, 1134s, 1082s, 1054m, 1031m. HRMS-ESI: calcd for $C_{24}H_{29}N_3NaO_4S_3$ $[M + Na]^+$ 542.12124, found 542.12083. R_f (deactivated silica gel, CH_2Cl_2/hexane/Et_3N 3.5/1.5/0.1): 0.64.

■ ASSOCIATED CONTENT

Supporting Information

Brief note on the instability of *des*-methyl **5**; copies of ^{1}H NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge financial support from the Institute of Organic Chemistry (University of Zurich) and the Swiss National Science Foundation.

■ REFERENCES

(1) For representative reviews of fluorescent chemosensors and probes, see: (a) Formica, M.; Fusi, V.; Giorgi, L.; Micheloni, M. *Coord. Chem. Rev.* **2012**, *256*, 170−192. (b) Cho, D.-G.; Sessler, J. L. *Chem. Soc. Rev.* **2009**, *38*, 1647−1662. (c) Tsukanov, A. V; Dubanosov, A. D.; Bren, V. A.; Minkin, V. I. *Chem. Heterocycl. Compd.* **2008**, *44*, 899−923. (d) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551−8588. (e) Bell, T. W.; Hext, N. M. *Chem. Soc. Rev.* **2004**, *33*, 589−598. (f) Rurack, K.; Resch-Genger, U. *Chem. Soc. Rev.* **2002**, *31*, 116−127. (g) Bren, V. A. *Russ. Chem. Rev.* **2001**, *70*, 1017−1036. (h) de Silva, A. P.; Gunarathe, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 515.

(2) We differentiate between chemosensors, which provide a signal in response to a reversible binding event, and chemodosimeters, which provide a signal in response to a transformation that is irreversible. For an early articulation of this important distinction, see: *Fluorescent Chemosensors for Ion and Molecule Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1993.

(3) For systematic evaluations of intramolecular PET in aminoalkyl fluorophores, including variation of quenching efficiency with chain length, see: (a) Shizuka, H.; Nakamura, M.; Morita, T. *J. Phys. Chem.* **1979**, *83*, 2019−2024. (b) Vanderawera, P.; DeSchryver, F. C.; Weller, A.; Winnik, M. A.; Zachariasse, K. A. *J. Phys. Chem.* **1984**, *88*, 2964−2970. (c) Beeson, J. C.; Huston, M. E.; Pollard, D. A.; Venkatachalam, T. K.; Czarnik, A. W. *J. Fluoresc.* **1993**, *3*, 65−67. For overviews of PET-based fluorescent chemosensors, see: (d) Bissell, R. A.; de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G. E. M.; McCoy, C. P.; Sandanayake, K. R. A. S. *Top. Curr. Chem.* **1993**, *168*, 223−264. (e) da Silva, A. P.; Uchiyama, S. *Top. Curr. Chem.* **2011**, *300*, 1−28.

(4) Other signaling mechanisms, such as modulation of intramolecular charge transfer (ICT), are also important. For an overview, see: Demchenko, A. P. *Introduction to Fluorescence Sensing*; Springer Science: London, 2009; Chapter 6. See also ref 1.

(5) PET-based chemodosimeters based on phosphorus have been reported in which phosphine oxidation to the phosphine oxide leads to significant fluorescence enhancement. For examples, see: (a) Akasaka, K.; Suzuki, T.; Ohrui, H.; Meguro, H. *Anal. Lett.* **1987**, *20*, 731−745. (b) Onoda, M.; Uchiyama, S.; Endo, A.; Tokuyama, H.; Santa, T.; Imai, K. *Org. Lett.* **2003**, *5*, 1459−1461. (c) Lemieux, G. A.; de Graffenried, C. L.; Bertozzi, C. R. *J. Am. Chem. Soc.* **2003**, *125*, 4708−4709. (d) Fang, A. G. Development of novel fluorescent chemosensors. Ph.D. Thesis, University of California, San Diego, La Jolla, CA, 2003. We have described the use of sulfoxide fluorophores as chemodosimeters for peroxide detection and noted that they also respond to added metal ion: (e) Malashikhin, S. A.; Finney, N. S. *J. Am. Chem. Soc.* **2008**, *130*, 12846−12847.

(6) For reviews of anion coordination by ureas/thioureas, see: (a) Gunnlaugsson, T.; Ali, H. D. P.; Glynn, M.; Kruger, P. E.; Hussey, G. M.; Pfeffer, F. M.; Santos, C. M. G.; Tierney, J. *J. Fluoresc.* **2005**, *15*, 287−299. (b) Amendola, V.; Bonizzoni, M.; Esteban-Gomez, D.; Fabbrizzi, L.; Licchelli, M.; Sancenon, F.; Taglietti, A. *Coord. Chem. Rev.* **2006**, *250*, 1451−1470. (c) Gunnlaugson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094−3117. (d) Li, A.-F.; Wang, J.-H.; Wang, F.; Jiang, Y.-B. *Chem. Soc. Rev.* **2010**, *39*, 3729−3745.

(7) Fluorescent chemodosimeters based on thioureas have been reported. These most frequently involve desulfurization by Hg^{2+}; for representative examples, see especially ref 1a,b. There are two reports of Hg^{2+}-responsive fluorescent chemosensors based on aminomethylanthracene thioureas in which PET has been stated as the operant signaling mechanism: (a) Profatilova, I. A.; Bumber, A. A.; Tolpygin, I. E.; Rybalkin, V. P.; Gribanova, T. N.; Mikhailov, I. E.; Bren, V. A. *Russ. J. Gen. Chem.* **2005**, *75*, 1774−1781. (b) Tolpygin, I. E.; Shepelenko, E. N.; Revinskii, Y. V.; Tsukanov, A. V.; Dubonosov, A. D.; Bren, V. A.; Minkin, V. I. *Russ. J. Gen. Chem.* **2010**, *80*, 756−770. Reversibility is not demonstrated, and the possibility of desulfurization is not addressed in these reports. Further, recent transient absorption measurements reveal no evidence of a PET process in the aminomethylanthracene phenylthiourea: (c) Del Giacco, T.; Carlotti, B.; De Solis, S.; Barbafina, A.; Elisei, F. *Phys. Chem. Chem. Phys.* **2010**, *12*, 8062−8070.

(8) Metal coordination by thioureas with an *N*-acyl group as an additional chelating element has been studied in depth. For a review, see: (a) Koch, K. R. *Coord. Chem. Rev.* **2001**, *216−217*, 473−488. The use of an *N*-naphthyl ferrocenyl thiosemicarbazone has been reported

as metal-responsive electrochemical and fluorescent chemosensor. Reversibility is not demonstrated, and the direct conjugation of the thiourea and the naphthyl fragment precludes involvement of PET: (b) Liu, W.; Li, X.; Li, Z.; Zhang, M.; Song, M. *Inorg. Chem. Commun.* **2007**, *10*, 1485−1488. During the course of this work, an aminomethylpyrene-derived *N*-acylthiourea was reported as a fluorescent chemosensor for Hg^{2+} in 1:1 H_2O/CH_3CN: (c) Li, X. L.; He, Y. W.; Yang, S. I. *Bull. Korean Chem. Soc.* **2011**, *32*, 338−340 While reversibility of the Hg^{2+} response is demonstrated, in light of ref 7c it is unlikely that this chemosensor relies on PET quenching..

(9) By "well-defined", we mean examples in which metal binding has been shown to be reversible (a requirement for a molecule being denoted as a chemosensor) and in which the mechanism of fluorescence enhancement has been evaluated rather than merely asserted.

(10) Naphthalimides are versatile and hydrolytically stable fluorophores. For an overview of their properties and applications, see: Duke, R. M.; Veale, E. B.; Pfeffer, F. M.; Kruger, P. E.; Gunnlaugson, T. *Chem. Soc. Rev.* **2010**, *39*, 3936−3953 and references therein.

(11) For a review of bis(2-pyridylmethyl) amines in molecular recognition, see: (a) Kruppa, M.; Koenig, B. *Chem. Rev.* **2006**, *106*, 3520−3560. For metal ion coordination by 7-aza-1,4-dithiacyclononane, see: (b) McAuley, A.; Subramanian, S. *Inorg. Chem.* **1990**, *29*, 2830−2837. (c) Blake, A. J.; Danks, J. P.; Fallis, I. A.; Harrison, A.; Li, W.-S.; Parsons, S.; Ross, S. A.; Whittaker, G.; Schroeder, M. *J. Chem. Soc., Dalton Trans.* **1998**, 3969−3976. (d) van de Water, L. G. A.; ten Hoonte, F.; Driessen, W. L.; Reedijk, J.; Sherrington, D. C. *Inorg. Chim. Acta* **2000**, *303*, 77−85.

(12) The driving force for PET, ΔG_{ET}, is estimated as follows. The reduction potential of **4** is taken as the absolute LUMO energy of the naphthalimide (−1.80 V vs Fc/Fc^+). The energy corresponding to the (0,0) band of the naphthalimide (396 nm; E_{00} = 3.13 eV) is used to estimate the absolute energy of the naphthalimide HOMO (+1.33 eV). The E^0 value for oxidation of **1** is taken as the absolute HOMO energy of the thiourea fragment (+0.90 V). Assuming that the energy levels of the singly occupied orbitals in the naphthalimide excited state are the same as the ground-state HOMO and LUMO energies, the difference between the HOMO values of the thiourea and the naphthalimide represents the driving force for electron transfer; in the present case, $\Delta G_{ET} \approx -0.43$ eV = 9.91 kcal/mol. While this is an approximation, it is well established; see: *Handbook of Photochemistry*; Montalti, M., Credo, A., Prodi, L., Gandolfi, M. T., Eds.; CRC Press: Boca Raton, FL, 2006.

(13) We observe increased emission from **1** in CH_2Cl_2 in response to added $HgCl_2$, indicating that the thiourea fragment is (as expected) capable of binding to Hg^{2+} in nonpolar solvent. However, this intrinsic affinity is apparently insufficient to compete with solvation by CH_3OH.

(14) The recurring deflection at ∼450 nm is a Wood's anomaly characteristic of our fluorimeter.

(15) Binding is illustrated with $ZnCl_2$ to allow inclusion of **1**, which does not bind $HgCl_2$ in CH_3OH.[13]

(16) Binding constants were determined by nonlinear least-squares fitting of plots of emission intensity versus log[M] using the program Prism3 (Graphpad, Inc., San Diego, CA). Fitting was consistent with formation of 1:1 M·L complexes and has been confirmed for titrations of **5** with $ZnCl_2$ and $HgCl_2$, and we have obtained the X-ray structure of a 1:1 complex between $ZnCl_2$ and a ligand related to **7**. While other binding events have been characterized in less detail, variation in the stoichiometry does not affect any conclusions reached here.

(17) We have found that **1** and **7** can be recovered untransformed following exposure to Zn^{2+} and Hg^{2+} for several hours in CH_3OH. This excludes the possibility that fluorescence enhancements are the result of desulfurization of the thiourea. The addition of chelating agents (EDTA or dipicolyl amine) reverses the observed fluorescence enhancements.

(18) For an overview of optical Hg^{2+} detections, see: (a) Nolan, E. M.; Lippard, S. J. *Chem. Rev.* **2008**, *108*, 3443−3480. For an overview of fluorescence detection of Zn^{2+}, see: (b) Jiang, P.; Guo, Z. *Coord. Chem. Rev.* **2004**, *248*, 205−229.

(19) This was not a foregone conclusion, given that the barriers to thiourea C−N bond rotation in **5**−**8** are likely only ∼11−13 kcal/mol. See: (a) Sullivan, R. H.; Price, E. *Org. Magn. Reson.* **1975**, *7*, 143−150. (b) Haushalter, K. A.; Lau, J.; Roberts, J. D. *J. Am. Chem. Soc.* **1996**, *118*, 8891−8896.

(20) The observed binding profiles for Ag^+ and Pb^{2+} are intriguing, but their origin is presently unclear. These responses dissipate completely in aqueous media.

(21) In *N*-methylthiourea, the *s-cis* isomer is favored over the *s-trans* isomer by a factor of 2.1−2.3 in D_2O, and this ratio is reported as being "higher" in methanol. This is similar to the 1.4- to 4-fold variance of $(I/I_0)_{max}$ seen between **5** and **7**. See: Tompa, A. S.; Barefoot, R. D.; Price, E. *J. Phys. Chem.* **1969**, *73*, 435−438.

(22) A further test of this proposal would be comparison of the $(I/I_0)_{max}$ values for metal titrations of **5** and its *des*-methyl analogue. Unfortunately, *des*-methyl **5** is unstable due to elimination/carbodiimide formation. See the Supporting Information.

(23) At $[7]_0$ = 33 nM (999:1 H_2O/CH_3OH) summation of five emission scans at each Hg^{2+} concentration is required for reliable measurement. Half-maximum response still occurs at $[Hg^{2+}]_{total} = [7]_0$. Using K_d(apparent) = $[Hg^{2+}]\cdot[7]/[7\cdot Hg^{2+}]$, at half-maximum $[7] = [7\cdot Hg^{2+}]$ and K_d(apparent) = $[Hg^{2+}] = [Hg^{2+}]_{total} - [7\cdot Hg^{2+}] = [Hg^{2+}]_{total} - 0.5 \times [7]_0 = 0.5 \times [7]_0$ = 17 nM. Thus, log K_d is at least −7.8.

(24) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Springer: New York, 2006.

(25) Berlman, B. I. *Handbook of Fluorescent Spectra*; Academic Press: New York, 1965.

(26) Eisenführ, A.; Arora, P. S.; Sengle, G.; Takaoka, L. R.; Nowick, J. S.; Famulok, M. *Bioorg. Med. Chem.* **2003**, *11*, 235−249.

(27) Jentzsch, E.; Mokhir, A. *Inorg. Chem.* **2009**, *48*, 9593−9595.

(28) Montero, A.; Goya, P.; Jagerovic, N.; Callado, L. F.; Meana, J. J.; Girón, R.; Goicoechea, C.; Martín, M. I. *Bioorg. Med. Chem.* **2002**, *10*, 1009−1018.

(29) West, C. W.; Estiarte, M. A.; Rich, D. H. *Org. Lett.* **2001**, *3*, 1205−1208.

(30) van de Water, L. G. A.; Hoonte, F.; Driessen, W. L.; Reedijk, J.; Sherrington, D. C. *Inorg. Chim. Acta* **2000**, *303*, 77−85.

(31) McAuley, A.; Subramanian, S. *Inorg. Chem.* **1990**, *29*, 2830−2837.