A Novel Fluorescent Monomer for the Selective Detection of Phenols and Anilines

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Received October 19, 1998

The detection of analytes utilizing the fluorescence response of synthetic molecular probes is a growing area of academic and industrial research.[1−3] The ultimate goal of this work is to obtain a chemosensor with quantitative, real-time optical response and exceptional selectivity for a particular analyte or class of analytes.[1] Alcohols and amines are two classes of analytes that have recently received a great deal of attention in the area of fluorescence detection because of the prevalence of hydroxy- and amine-containing molecules in biology and the extensive use of alcohols and amines in industrial applications.

A number of novel solution-based fluorescent probes with sensitivity for alcohols have recently been reported. For example, Ueno and co-workers[4−6] have developed a series of fluorescent cyclodextrins for the host−guest sensing of long-chain and biologically relevant alcohols. Kumar and co-workers have developed a pyridine/pyrene-based fluorescent probe that is quenched by protic analytes such as phenol, aniline, and water via a hydrogen-bonding mechanism.[7,8] Although amines are generally known to quench the fluorescence of a large number of commercial dyes, a number of novel fluorophores with sensitivity to amine-containing analytes have also been recently developed. For example, Shinkai and co-workers developed a pyrene-based receptor for the detection of barbital via hydrogen-bonding interactions.[9] Sun et al. used methylene diphenylene diisocyanate as a fluorescent probe to monitor the conversion of amines and alcohols during polyurethane formation.[10] More recently, Fabbrizzi and co-workers developed a fluorescent dizinc anthracene−octamine complex that is quenched by the binding of imidazole and histidine.[11]

Although these novel sensing molecules have been shown to operate well in solution, in terms of practicality a solid-state sensor is often more desirable. A reversible solid-state chemosensor minimizes sample contamination (especially for in vivo applications) and, after regeneration of the sensing element, can be used again. One of the most common approaches for fabricating solid-state alcohol and amine sensors is to immobilize fluorescent probe molecules onto the end of a fiber optic cable.[12−15] Alternatively, solid-state alcohol and amine sensors have also been fabricated by embedding the desired fluorophores into a porous solid material. For example, sol−gel chemistry has been used to immobilize synthetic dyes as well as fluorescent biological sensing systems to fabricate silica-based sensors capable of detecting alcohols in water.[16,17] In terms of organic solid-state sensors, a number of researchers have designed polymer-based alcohol sensors by doping poly(vinyl chloride) films with fluorescein-, stillbene-, and azobenzene-based dyes.[18−20] This same technique has been used to produce polymer-based thiamine sensors.[21] Finally, Orellana and co-workers have recently synthesized a series of fluorescent, pyrazine-based dyes that are not only alcohol sensitive but can also be easily attached to fibers or solid supports.[22] In this system, fluorescence quenching is accomplished via a hydrogen-bonding interaction with the alcohols.

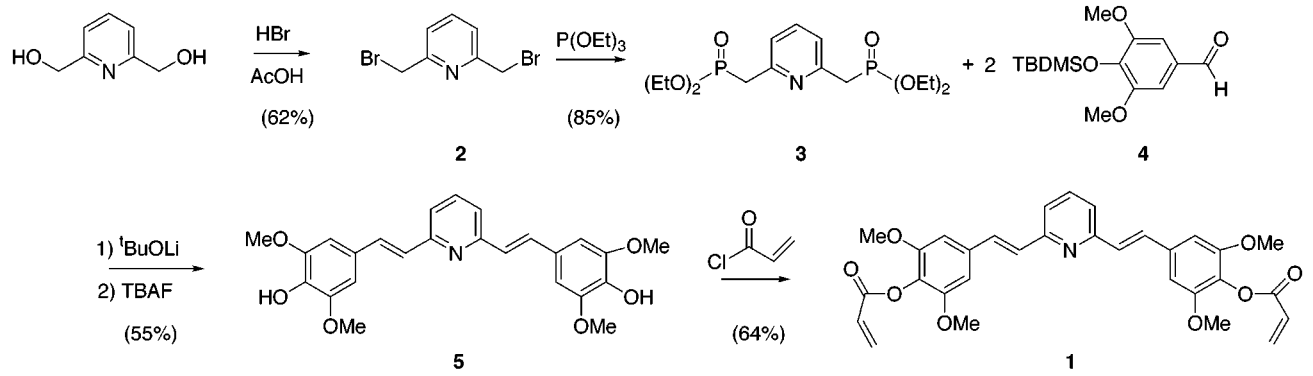
We have developed a new polymerizable fluorescent probe (**1**) that is quenched selectively by aromatic alcohols and amines, even in the presence of their aliphatic analogues, oxygen, and water. This selective quenching occurs with **1** dissolved in nonpolar solvents such as benzene or cross-linked inside a polymethacrylate matrix. Monomer **1** contains a central pyridine ring similar to Kumar's fluorophore;[7,8] however, it has a different conjugated core architecture and can also participate in radical copolymerizations with conventional monomers. This novel fluorophore architecture leads to a different mechanism of fluorescence quenching from that of Kumar's fluorophore and also to a high degree of analyte selectivity.

Monomer **1** was synthesized as shown in Scheme 1. 2,6-Pyridinedimethanol was brominated with HBr in acetic acid[23] to afford 2,6-bis(bromomethyl)pyridine (**2**).[24] This compound was subsequently reacted with triethyl phosphite to yield the corresponding phosphonate ester (**3**),[25] which then underwent a Wadsworth−Emmons condensation[26] with 2 equiv of TBDMS-protected syringaldehyde (**4**).[27] Removal of the silyl ether protecting

(1) Czarnik, A. W. *Chem. Biol.* **1995**, *2*(7), 423.
(2) Mahutte, C. K. *Clin. Biochem.* **1998**, *31*(3), 119.
(3) Johnson, I. *Histochem. J.* **1998**, *30*(3), 123.
(4) Ueno, A.; Minato, S.; Osa, T. *Anal. Chem.* **1992**, *64*, 1154.
(5) Ueno, A.; Minato, S.; Osa, T. *Anal. Chem.* **1992**, *64*, 2562.
(6) Hamasaki, K.; Ueno, A.; Toda, F. *J. Chem. Soc., Chem. Commun.* **1993**, 331.
(7) Kumar, C. V. *J. Chem. Soc., Chem. Commun.* **1993**, 772.
(8) Kumar, C. V.; Tolosa, L. M. *J. Photochem. Photobiol. A: Chem.* **1994**, *78*, 63.
(9) Aoki, I.; Harada, T.; Sakaki, T.; Kawahara, Y.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1992**, 1341.
(10) Sun, X.-D.; Sung, C. S. P. *Polym. Prepr., Am. Chem. Soc. Div. Polym. Chem.* **1994**, *35*(1), 435.
(11) Fabbrizzi, L.; Francese, G.; Licchelli, M.; Perotti, A.; Taglietti, A. *Chem. Commun.* **1997**, 581.
(12) Wolfbeis, O. S.; Posch, H. E.; *Fresenius' Z. Anal. Chem.* **1998**, *332*(3), 255.
(13) White, J.; Kauer, J. S.; Dickinson, T. A.; Walt, D. R. *Anal. Chem.* **1996**, *68*(13), 2191.
(14) Dickinson, T. A.; White, J.; Kauer, J. S.; Walt, D. R. *Nature* **1996**, *382*, 697.
(15) Charlesworth, J. M.; McDonald, C. A. *Sens. Actuators B* **1992**, *B8*(2), 137.
(16) Simon, D. N.; Czolk, R.; Ache, H. J. *Thin Solid Films* **1995**, *260*(1), 107.
(17) Williams, A. K.; Hupp, J. T. *J. Am. Chem. Soc.* **1998**, *120*(18), 4366.
(18) Zeng, H.-H.; Wang, K.-M.; Li, D.; Yu, R.-Q. *Talanta* **1994**, *41*(6), 969.
(19) Mohr, G. J.; Lehmann, F.; Grummt, U.-W.; Spichiger-Keller, U. E. *Anal. Chem. Acta* **1997**, *344*(3), 215.
(20) Mohr, G. J.; Spichiger-Keller, U. E. *Anal. Chim. Acta* **1997**, *351*(1−3), 189.
(21) He, H.; Uray, G.; Wolfbeis, O. S. *Anal. Lett.* **1992**, *25*(3), 405.
(22) Orellana, G.; Gomez-Carneros, A. M.; de Dios, C.; Garcia-Martinez, A. A.; Moreno-Bondi, M. C. *Anal. Chem.* **1995**, *67*(13), 2231.
(23) Baker, W.; Buggle, K. M.; McOmie, J. F. W.; Watkins, D. A. M. *J. Chem. Soc.* **1958**, 3594.
(24) Offerman, W.; Voegtle, F. *Synthesis* **1977**, 272.
(25) Kabachnic, M. I.; Polikarpv, Y.; Bodrin, G. V.; Bel'skii, F. I. *Bull. Acad. Sci. USSR Div. Chem. Sci. (Eng.)* **1988**, *37*, 800.
(26) Wadsworth, W. S. *Org. React.* **1977**, *25*, 73.

10.1021/jo982105e CCC: $18.00
Published on Web 04/30/1999

Scheme 1



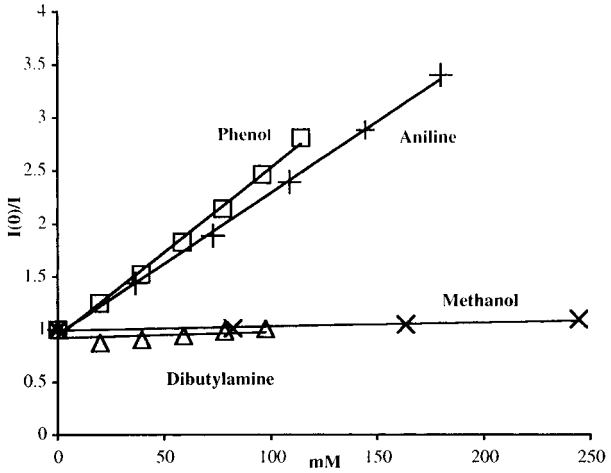


Figure 1. Stern−Volmer plots of solution quenching of **1** (90 μM, benzene): □, phenol; △, dibutylamine; +, aniline; ×, methanol.

groups on the resulting intermediate using tetra(*n*-butyl)-ammonium fluoride[28] gave the bisphenol **5**. Finally, acrylation of **5** with acryloyl chloride[29] afforded **1** in 19% overall yield.

Initial spectroscopic studies of **1** dissolved in benzene (90 μM) revealed that the monomer has characteristic absorption maxima at 309 and 348 nm, intense emission maxima at 395 and 420 nm when excited at 310 or 370 nm, and a fluorescence lifetime of 1.45 ns. The intensity ratio of the 395 and 420 nm emission bands (1.06:1.00) is largely invariant to monomer concentration below the millimolar level.

A wide variety of alcohols and amines were added to the 90 μM benzene solution of **1**, in concentrations of 10−100 mM, to test their quenching behavior. Anilines and phenols were found to act as strong quenchers with Stern−Volmer constants (K_{sv}) ranging from 13 to 18 M^{-1}, whereas aliphatic amines and alcohols were found to be very weakly quenching or effectively nonquenching (Figure 1). The quenching efficiencies of the added species can be correlated to their relative ease of oxidation, as ranked by their oxidation potentials ($E_{1/2}$) measured in a common solvent[30−32] and compared against a common reference electrode (Table 1). This trend is consistent with

Table 1. Quenching of 1 (90 μm in Benzene) by Various Alcohols and Amines

analyte	K_{sv}[a] (M^{-1})	rel oxidation $E_{1/2}$ values (V) vs SCE[b]
N,*N*-dimethylaniline	18	0.68
N-methylaniline	16	0.78
m-toluidine	15	
aniline	13	0.86
phenol	17	1.37
m-cresol	17	1.28
p-cresol	15	1.17
indole	8.9	
benzyl alcohol	1.6	>2.33
ethanol	1.1	2.87
cyclohexanol	0.7	>2.33
t-butanol	0.6	2.86
triethylamine	1.3	0.99
dibutylamine	0.5	1.20
butylamine	0.2	1.52

[a] Measured in benzene solution. [b] Derived from tables of reported oxidation $E_{1/2}$ values measured in acetonitrile. See refs 30−32. It was not possible to obtain oxidation potentials of the compounds in benzene solution.

an electron-transfer quenching mechanism as described by Rehm and Weller, in which the excited state of **1** is able to spontaneously oxidize the quenching species and undergo subsequent nonradiative decay.[33] Within this trend, it should be noted that the phenols and the aliphatic amines are comparable in oxidizability; however, the phenols are extremely good quenchers whereas the aliphatic amines are almost nonquenching. This apparent anomaly can be rationalized by the fact that phenols are somewhat acidic ($pK_a \approx 10$) whereas aliphatic

(27) Cushman, M.; Nagatarathnam, D.; Gopal, D.; He, H.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1992**, *35*(12), 2293.

(28) Corey, E. J.; Venkateswarlu, A. J. *J. Am. Chem. Soc.* **1972**, *94*, 6190.

(29) Broer, D. J.; Boven, J.; Mol. G. N.; Challa, G. *Makromol. Chem.* **1989**, *190*, 2255.

(30) For tabulated oxidation $E_{1/2}$ values of amines, see: Reed, R. C.; Wightman, R. M. In *Encyclopedia of Electrochemistry of the Elements, Organic Section*; Bard, A. J., Lund, H., Eds.; Marcel Dekker: New York, 1978; Vol. XV, Chapter XV-1, pp 4−30.

(31) For tabulated oxidation $E_{1/2}$ values of alcohols, see: Parker, V. D.; Sundholm, G.; Svanholm, U.; Rolán, A.; Hammerich, O. In *Encyclopedia of Electrochemistry of the Elements, Organic Section*; Bard, A. J., Lund, H., Eds.; Marcel Dekker: New York, 1978; Vol. XI, Chapter XI-2, pp 182−183 and 188.

(32) $E_{1/2}$ values reported in the literature are typically those measured in water, CH_3CN, or propylene carbonate solution using 0.1 or 0.15 M supporting electrolyte and a Pt anode. $E_{1/2}$ values reported for compounds in the same solvent are also often cited against many different reference electrodes. To provide a *qualitiative* ranking of the oxidizability of the various analytes, oxidation $E_{1/2}$ values in CH_3CN of the compounds were obtained from the literature and converted to values reported against a common reference electrode (SCE). The oxidation $E_{1/2}$ value for **1** in acetonitrile was measured to be 1.92 V vs SCE. See the Supporting Information for details.

(33) (a) Rehm, D.; Weller, A. *Israel J. Chem.* **1970**, *8*, 259. (b) Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper Collins: New York, 1987; pp 231−232.

Table 2. Quenching of 1 (90 μm in Benzene) by Various Protic Compounds and Common Solvents

analyte	K_{sv}[a] (M^{-1})	rel oxidation $E_{1/2}$ values (V) vs SCE[b]
hexanethiol	0.5	~1.82
acetic acid	2.4	1.58
anisole	0.2	
DMSO	1.0	
acetone	0.2	
2-butanone	0.3	

[a] Measured in benzene solution. [b] Derived from tables of reported oxidation $E_{1/2}$ values measured in acetonitrile. See refs 32, 36, and 37. It was not possible to obtain oxidation potentials of the compounds in benzene solution.

amines are effectively nonacidic ($pK_a \approx 35$). Thus, phenols can partially donate a proton (e.g., hydrogen bond) to the basic pyridine nitrogen on the fluorophore. This binding of the phenolic hydrogen by the pyridine moiety of the fluorophore will lead to more partial negative charge character on the phenol oxygen and thus lower the effective oxidation potential. It has been shown that deprotonation of phenols lowers their oxidation potentials by 0.5−0.7 V.[34] Consequently, phenols act as better oxidative quenchers than would be expected from their neutral oxidation potentials.

To determine whether protonation or hydrogen bonding alone can lead to quenching of **1**, a series of control experiments with protic species of differing pK_a values and hydrogen-bonding abilities were tested (Table 2). Hexanethiol, which is of comparable acidity to phenol but a poor hydrogen-bond donor[35] with a much higher oxidation potential,[32,36] showed negligible quenching. The relatively low K_{sv} values measured for the aliphatic alcohols, which are good hydrogen-bond donors[35] but not very acidic, suggest that hydrogen bonding is only a very minor contributor to quenching. Acetic acid, a much stronger acid ($pK_a \approx 4.7$) and a better hydrogen-bond donor than the phenols,[35] was found to be a weak quencher with a slightly larger K_{sv} (2.4 M^{-1}) than the aliphatic alcohols. It should be noted that protonated and deprotonated acetic acid have approximately the same oxidation potential,[37] which is much higher than that of the other effective quenchers. In fact, it is comparable to the oxidation potential of nonquenchers such as butylamine. This suggests that for acetic acid the slight quenching observed likely arises from protonation of the fluorophore rather than electron transfer from the quencher. In contrast, the strong quenching shown by *N*,*N*-dimethylaniline, which has a low oxidation potential[30] and no labile protons, demonstrates that electron transfer is a much more effective quenching mechanism than proton transfer or hydrogen bonding. Thus, it appears that the mechanism for the selective quenching of **1** is primarily dependent on the ease of oxidation of the quenching species, with proton donation from sufficiently acidic analytes providing a small degree of quenching. To establish conditions under which the fluorophore might be used for sensor applications, common organic solvents that are miscible with benzene (e.g., acetone, DMSO, 2-butanone) were also tested and found to be relatively nonquenching with respect to **1**.

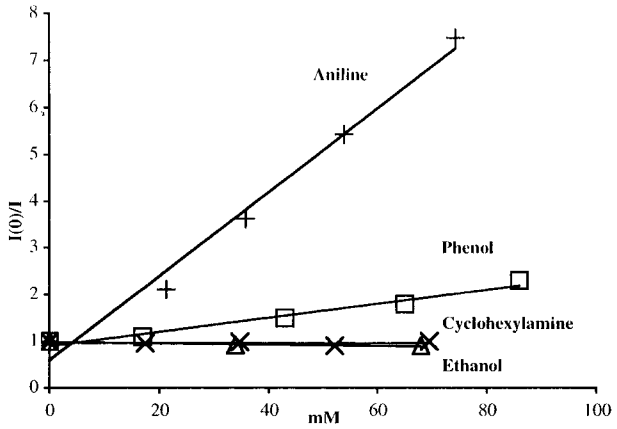


Figure 2. Stern−Volmer plots of quenching of poly(**1**-co-EGDMA), cyclohexane solutions: □, phenol; △, ethanol; +, aniline; ×, cyclohexylamine.

Table 3. Quenching of Poly(1-*co*-EGDMA) Beads Immersed in Cyclohexane

analyte	K_{sv} (M^{-1})
aniline	70
N-methylaniline	33
N,*N*-dimethylaniline	21
indole	51
cyclohexylamine	0
phenol	15
m-cresol	16
methanol	1.0
ethanol	0

To demonstrate that **1** could be used in a solid-state fluorescent sensor, **1** (0.6 wt %) was cross-linked into macroporous poly(EGDMA) beads (30 μm). The beads were then adhered onto quartz slides using a thin film of transparent cyanoacrylate resin (i.e., SuperGlue) for systematic solid-state fluorimetry studies (Table 3). It was not possible to use benzene for these studies because benzene tended to swell the glue resin, causing the beads to detach from the plates. Instead, the initial solid-state studies were performed primarily with the adhered fluorescent beads immersed in cyclohexane. Later studies were performed with the beads held on to the end of a fiber optic bundle. Similar K_{sv} values were found with the slide and the fiber optic methods.

As can be seen from Table 3 and Figure 2, the quenching trends observed with **1** in solution (Figure 1) also hold for **1** in the cross-linked polymer, showing again the selective quenching of **1** with phenols and anilines over aliphatic alcohols and amines. Comparison of the quenching of **1** dissolved in benzene and of cross-linked **1** immersed in benzene shows that the response in the poly(EGDMA) matrix is much lower than that in solution but still quite detectable. Furthermore, the response of fluorescent polymer to the marginally quenching or nonquenching analytes drops to zero; thus the analyte selectivity of **1** is enhanced. In the case of the anilines, the fluorescent polymer shows increased selectivity by being able to better distinguish between aniline, *N*-methylaniline, and *N*,*N*-dimethylaniline. The trend in the K_{sv} values of the three aniline derivatives for the fluorophore in the cross-linked polymer was found to be

(34) Weinberg N. L.; Weinberg H. R. *Chem. Rev.* **1968**, *68*, 449.
(35) Epshtein, L. M. *Russ. Chem. Rev.* **1979**, *48*(9), 854.
(36) Chambers, J. Q. In *Encyclopedia of Electrochemistry of the Elements, Organic Section*; Bard, A. J., Lund, H., Eds.; Marcel Dekker: New York, 1978; Vol. XII, Chapter XII-3, p 415.
(37) Eberson, L.; Nyberg, K. In *Encyclopedia of Electrochemistry of the Elements, Organic Section*; Bard, A. J., Lund, H., Eds.; Marcel Dekker: New York, 1978; Vol. XII, Chapter XII-2, p 266.

Table 4. Quenching of Poly(1-*co*-EGDMA) Beads by Phenols in Different Solvents

analyte	solvent	K_{sv} (M^{-1})
phenol	benzene	5.0
phenol	cyclohexane	15
phenol	1:1 ethanol/water	3.0
m-cresol	cyclohexane	17
m-cresol	ethanol	3.0

opposite to that found with **1** in solution and also the opposite to that predicted by their oxidation potentials according to the Rehm−Weller equation.[33] As shown in the first three entries of Table 3, aniline was found to be a more efficient quencher of **1** in the polymer than either *N*-methylaniline or *N*,*N*-dimethylaniline; however, *N*,*N*-dimethylaniline is more easily oxidized than either *N*-methylaniline or aniline.[30] This reversed trend may be due to the more substituted, and thus sterically hindered, amine groups being less able to approach the fluorophore in the micropores of the cross-linked network, thereby reducing the efficiency of electron transfer. The fluorescence quenching of **1** in the polymer matrix was found to be reversible upon exposure to fresh solvent, and the recovered polymer beads can be used for analyte detection with sensitivity comparable to that of fresh beads.

The nature of the solvent was found to have a strong effect on the quenching behavior of **1** in the polymer. The quenching of the fluorescence from the polymer beads by phenols was studied in a series of solvents including benzene, cyclohexane, ethanol, and 1:1 ethanol/water (Table 4). With the same analyte, the K_{sv} values of the poly(**1**-*co*-EGDMA) beads immersed in cyclohexane are approximately three times larger than those observed in ethanol or benzene and five to eight times greater than those observed in ethanol/water. The initial reductions in absolute fluorescence intensity upon immersing the polymer in pure cyclohexane and in pure water were comparable, however (80% and 84% drops, respectively). One possible explanation for this phenomenon is that different solvents may be able to penetrate and swell the cross-linked poly(EGDMA) matrix of the beads better than others. In polymer chemistry, it is well-known that different solvents can swell polymer gels to different degrees. This phenomenon would allow some solvents to cause more dramatic changes in the local microenvironment around the immobilized fluorophores than others or to transport quenchers more efficiently to the fluorophores than other solvents. Preliminary photophysical studies also revealed that the fluorescence lifetime of **1** changes depending on the environment around the fluorophore. For example, the fluorescence lifetime of **1** was found to be 1.45 ns in 90 μm benzene solution, 2.86 ns in the dry cross-linked polymer, and 2.49 ns when the polymer beads were wetted by benzene. This is a reflection of the sensitivity of the excited state of the fluorophore to its microenvironment. Unfortunately, monomer **1** is not soluble in cyclohexane or in ethanol/water, so it was not possible to determine whether this solvent dependence of quenching sensitivity also holds true in solution.

In summary, a novel polymerizable fluorophore has been synthesized that is highly selective for the detection of phenols and anilines. The selectivity of this fluorescent probe is comparable in both solution and the solid polymer. Sensing can be performed in the presence of air and ambient moisture. The mechanism for the selective fluorescence detection appears to be primarily based on the ease of electron transfer from the organic analyte, with partial proton donation from sufficiently acidic analytes providing a small degree of quenching. Incorporation of the fluorescent monomer into a polymer allows for fluorescence detection in a wide variety of solvents including alcohol/water. The ability to sense in aqueous and protic environments makes this fluorophore/polymer combination potentially useful for a wide range of applications from probing of contaminated wastewater to monitoring of biological systems. Ongoing research with **1** includes incorporation into an imprinted polymer matrix to further increase analyte selectivity.

Experimental Section

General Considerations. All synthetic manipulations were performed using standard Schlenk line techniques. Nitrogen was purified by passage through columns of Q-5 catalyst (Engelhard) and 13X molecular sieves (Aldrich). All reactions were performed under nitrogen flush. All reagents and anhydrous *N*,*N*-dimethylformamide (ACS certified) were purchased from the Aldrich Chemical Co., with the exception of imidazole, which purchased from Fisher Scientific. Tetrahydrofuran (THF) was purchased from Fisher and distilled from sodium/benzophenone ketyl prior to use. Reaction mixtures and chromatography fractions were monitored with EM Science 250 μm silica gel F_{254} plates. Column chromatography was performed using 40 μm silica gel purchased from J. T Baker and Optima grade solvents from Fisher Scientific. Fluorescence quenching experiments were performed in air, using ACS Spectroanalyzed grade benzene and cyclohexane purchased from Fisher. Analytes tested as quenchers were all reagent grade or higher. ^{1}H and ^{13}C NMR spectra were acquired at 500 and 125 MHz, respectively, using acetone-d_6 as the solvent. FT-IR spectra were taken on compounds cast from chloroform solutions onto NaCl plates. UV−vis absorption spectra were taken of the compounds as dilute benzene solutions. Fluorescence studies were performed using an ISA Spex Fluoromax 2 photon-counting fluorimeter equipped with a magnetic stirrer in the sample compartment and a fiber optic attachment. Fluorescence lifetime measurements were performed at the Applications Laboratory of ISA Spex, Inc., using a Fluorolog-Tau3 spectrometer equipped with a double-grating monochromator for excitation and a single-grating emission monochromator. High-resolution and low-resolution mass spectral analyses were performed at the Mass Spectrometry Facility in the Chemistry Department at the University of California, Berkeley.

2,6-Bis(bromomethyl)pyridine (2).[24] A solution of 2,6-pyridinedimethanol (1.50 g, 10.8 mmol) in 30 wt % hydrobromic acid in acetic acid (22 mL) was heated at 100 °C for 1.5 h and then poured onto ice (20 mL) and neutralized with aqueous 1 M NaOH. The resulting precipitate was collected and recrystallized from 1:1 hexane/ethyl acetate to give 1.72 g of product (62% yield). ^{1}H NMR shifts were consistent with values previously reported.[24] ^{13}C NMR: δ 156.97, 138.26, 122.85, 33.57.

2,6-Bis(diethoxyphosphorylmethyl)pyridine (3).[25] A flask was charged with **2** (6.00 g, 22.6 mmol) and triethyl phosphite (8.28 g, 49.8 mmol) and fitted with a Dean−Stark trap and condenser. The solution was heated at 110 °C for 4 h while ethyl bromide was generated. After being cooled to ambient temperature, the reaction mixture was concentrated in vacuo to give 8.80 g (85% yield) of **3** as a viscous orange oil. (A portion was purified for analysis by flash chromatography using an increasing gradient of 2-propanol (3−10%) in chloroform as the eluent.) ^{1}H NMR: δ 7.65 (d, 1H), 7.29 (d, 2H), 4.03(q, 8H), 3.35 (d, 4H), 1.22 (t, 12H). ^{13}C NMR: δ 152.92, 136.47, 122.06, 61.44 (d, J_{C-P} = 26 Hz), 35.96 (d, J_{C-P} = 535 Hz), 15.71.

4-*tert*-Butyldimethylsilyloxy-3,5-dimethoxybenzaldehyde (4).[27] To a solution of syringaldehyde (7.10 g, 39.0 mmol) and imidazole (6.63 g, 97.4 mmol) in anhydrous DMF (30 mL) at 0 °C was added *tert*-butyldimethylsilyl chloride (8.81 g, 58.5 mmol) in dry DMF (15 mL). The reaction mixture was then heated at 60 °C for 4 h. After being quenched with water, the mixture was extracted into diethyl ether, washed with saturated

aqueous sodium bicarbonate, and dried over anhydrous Na_2SO_4. Removal of the solvent in vacuo afforded 9.20 g (90% yield) of pure **4** as an off-white, crystalline solid. ^{1}H NMR shifts were consistent with values reported in the literature.[27] ^{13}C NMR: δ 191.52, 171.17, 152.97, 131.00, 107.35, 56.30, 26.17, 1.45, −4.25.

2,6-Bis(2-(4-hydroxy-3,5-dimethoxyphenyl)vinyl)pyridine (5). To a nitrogen-filled flask was charged a 1 M THF solution of lithium *tert*-butoxide (32.6 mL, 32.6 mmol) and dry THF (20 mL). After the solution was cooled to 0 °C, crude **3** (6.17 g, 16.3 mmol) in dry THF (20 mL) was then added dropwise, and the reaction mixture was stirred for 0.5 h at 0 °C. To this mixture was then added a solution of **4** (9.20 g, 31.0 mmol) in dry THF (30 mL). The resulting reaction mixture was stirred at room temperature for 6 h and then cooled to 0 °C, upon which time a solution of tetrabutylammonium fluoride (12.60 g, 40.0 mmol) in dry THF (50 mL) was added dropwise. The resulting bright red solution was subsequently stirred at room temperature for 2 h and then quenched with aqueous 1 M HCl (100 mL). The mixture was neutralized (pH 7) with aqueous 1 M NaOH and extracted with dichloromethane (3 × 75 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Recrystallization of the crude product from ethanol gave 6.30 g of pure **5** (55% yield) as a yellow-green solid. Mp: 115−116 °C dec. ^{1}H NMR: δ 7.73 (d, 2H), 7.68 (t, 1H), 7.29 (d, 2H), 7.17 (d, 2H), 7.00 (s, 4H), 3.90 (s, 12H), 2.8 (broad s, 2H). ^{13}C NMR: δ 155.58, 147.98, 136.83, 132.92, 127.78, 126.75, 104.74, 55.72. IR (cm^{-1}): 3425, 1637, 1602, 1514, 1451, 1334, 1110, 750. EI LRMS: *m*/*z* 435, 167. EI HRMS: calcd for $C_{25}H_{25}NO_6$ *m*/*z* 435.1682, found 435.1689.

2,6-Bis(2-(4-acryloyloxy-3,5-dimethoxyphenyl)vinyl)pyridine (1). A solution of acryloyl chloride (0.45 g, 5.5 mmol) in dry THF (5 mL) was added dropwise to a flask containing a solution of **5** (1.00 g, 2.3 mmol) and triethylamine (0.96 mL, 6.9 mmol) in dry THF (30 mL) cooled to 0 °C. The resulting reaction mixture was then stirred at room temperature for 1 h, cooled to 0 °C, and quenched with 0.2 M aqueous HCl. After the solution was adjusted to pH 7 with aqueous 1 M NaOH, it was extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were then washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel using 1:1 ethyl acetate/hexane as the eluent to afford 0.80 g (64%) of **5** as a yellow, crystalline solid. Mp: 110−111 °C (polym). ^{1}H NMR: δ 7.83 (d, 2H), 7.76 (t, 1H), 7.39 (d, 2H), 7.37 (d, 2H), 7.08 (s, 4H), 6.54 (d of d, 2H), 6.42 (d, 2H), 6.08 (d of d, 2H), 3.89 (s, 12H). ^{13}C NMR: δ 164.03, 156.24, 153.59, 138.20, 136.40, 133.44, 132.95, 129.71, 129.64, 128.65, 121.85, 104.79, 56.63. FT-IR (cm^{-1}): 1743, 1631, 1594, 1452, 1249, 1130, 977, 806. EI LRMS: *m*/*z* 543, 489, 435. EI HRMS: calcd for $C_{31}H_{29}NO_8$ *m*/*z* 543.1893, found 543.1892. Anal. Calcd for $C_{31}H_{29}O_8N$: C, 68.50; H, 5.38; N, 2.58. Found: C, 68.15; H, 5.68; N, 2.57.

Synthesis of Cross-Linked Poly(1-*co*-EGDMA) Beads. A vial containing **1** (0.3 mg, 0.6 μmol) and $CHCl_3$ (0.55 mL) was charged with ethylene glycol dimethacrylate (495.0 mg, 2.50 mmol), 2,2′-azobisisobutyronitrile (5.0 mg, 0.030 mmol), and $CHCl_3$ (0.45 mL). The mixture was then cooled to at 0 °C and agitated by ultrasound while being purged with nitrogen for 5 min. The vial was sealed under nitrogen, and the polymerization mixture was stirred at 65 °C for 12 h. The resulting glassy polymer was ground by hand, passed as a $CHCl_3$ suspension through a 30 μm nylon mesh, and then dried in vacuo at 70 °C for 24 h.

Fluorescence Quenching Experiments. Fluorescence data were collected with the fluorophore excited at 310 and at 370 nm. Quenching behavior was not dependent upon the excitation wavelength.

Solution measurements were performed on 90 μM benzene solutions of **1** in quartz cuvettes containing a micro stirbar. Analytes were added to the stirred cuvettes via syringe. The fluorescence intensity at 395 nm was recorded 5−10 min after each successive addition. Fluorescence measurements were taken in the absence of analytes and then after analyte addition.

Solid-state quenching experiments were performed on 30 μm beads of poly(**1**-*co*-EGDMA) adhered onto quartz slides with SuperGlue. Each slide was wedged diagonally inside a quartz cuvette in the fluorimeter sample chamber to ensure fixed positions of the beads with respect to the detector. For each experiment, the cuvette was filled with 2.5 mL of solvent and stirred with a micro stirbar. Analyte addition and fluorescence monitoring were performed as before. The observed fluorescence behavior of the polymer beads was verified by later studies using beads held to the tip of a fiber optic bundle by tissue paper and a wire holder. Fluorescence measurements were taken with the fiber tip immersed in pure solvent and then in stirred analyte solutions of increasing concentration, allowing 10 min for equilibration prior to each measurement.

The Stern−Volmer quenching constants, K_{sv}, of the analytes were calculated by plotting the ratio of the initial fluorescence intensity (I_0) at the emission maximum over the observed fluorescence intensity in the presence of the added analyte (I), as a function of increasing analyte concentration.[7,8] Linear regression gives the K_{sv} value as the slope.

Fluorescence Lifetime Measurements. Fluorescence lifetimes of **1** in benzene solution (90 μm) and cross-linked in the polymer beads (dry and wetted by benzene) were measured by the Applications Laboratory at ISA Spex, Inc. The data were well described by a double-exponential decay law.

Acknowledgment. This research was supported primarily by a Cottrell Scholars Award of Research Corp. (CS0376). Additional support was provided by research gifts from the Raychem Corp. and the 3M Co. The authors are indebted to Dr. Salvatore Atzeni of ISA Spex, Inc., for measuring the fluorescence lifetimes of **1** in benzene and in the cross-linked polymer beads. We are also grateful to Dr. Olivier Buriez and Mr. Benjamin J. Gross for measuring the oxidation potential of **1** in CH_3CN.

Supporting Information Available: 500 MHz ^{1}H and 125 MHz ^{13}C NMR spectra of compounds **1** and **5**; reported oxidation $E_{1/2}$ values of the analytes, and the measured oxidation $E_{1/2}$ value of **1** in CH_3CN. This material is available free of charge via the Internet at http://pubs.acs.org.

JO982105E