Design of a Hydrophobic Fluorescent Probe: An Amide-Linked Bispyrenyl Alcohol

Frederick D. Lewis,* Yifan Zhang, and Robert L. Letsinger*

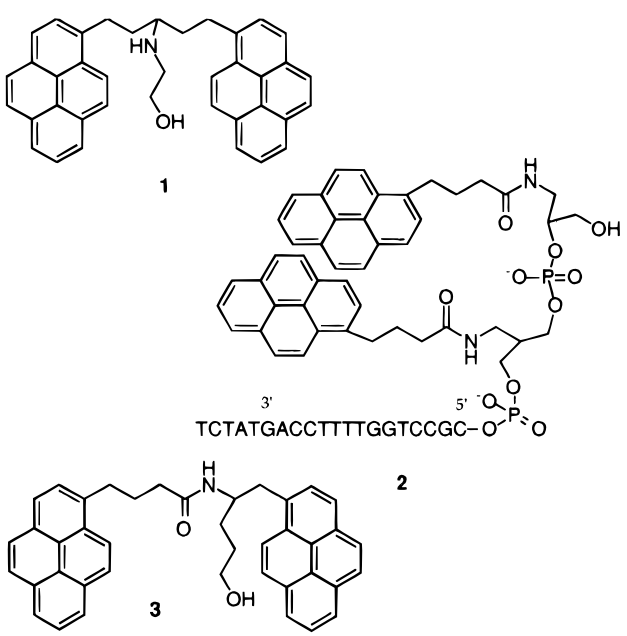
Department of Chemistry, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208

Received June 10, 1997

Molecules which are labeled with two or more pyrenyl chromophores can exhibit both monomer and excimer fluorescence.[1] The large spectral shift for pyrene excimer vs monomer emission and high fluorescence quantum yields make such molecules attractive as fluorescent probes and chemosensors.[2] The ratio of excimer to monomer fluorescence intensity (I_E/I_M) is dependent upon molecular structure, temperature, and solvent polarity. Zachariasee and co-workers[3] have investigated the chain-length dependence of I_E/I_M for α,ω-dipyrenylalkanes ($Py(CH_2)_nPy$, $n = 2-16, 22$) in hydrocarbon solvents. Both monomer and excimer fluorescence are observed for all chain lengths except $n = 7$, for which chain folding is thermodynamically unfavorable. The ratio I_E/I_M for dipyrenylalkanes is temperature dependent, increasing with increasing temperature at low temperatures and decreasing at higher temperatures, with a turnover region in between.[4] The ratio I_E/I_M for pyrene-labeled poly(ethylene oxide)s is also solvent-dependent.[5] Abnormally large values of I_E/I_M observed in methanol or water are attributed to ground-state pyrene association in these solvents.

We have employed stilbene and naphthalene excimer fluorescence to probe DNA duplex formation between complementary oligonucleotides, both of which possess covalently attached aromatic chromophores.[6] Our interest in developing a fluorescent probe of the sequence and environment for single- and double-stranded RNA and DNA led us to consider bispyrenyl molecules which could be covalently attached to an oligonucleotide. Whereas this concept is not novel, it has not been successfully executed. Kitamura et al.[7] have reported that the bispyrenyl derivative **1** (Chart 1) displays both monomer and excimer fluorescence in aqueous solution ($I_E/I_M \sim 1$) and suggested that it might be introduced into an oligonucleotide by the phosphoramidite method. Korshun et al.[8] prepared the bispryene−oligonucleotide conjugate **2** but observed only pyrene monomer fluorescence in aqueous solution. We report here the synthesis and solvent dependence of the absorption and fluorescence spectra of the bispyrene **3**. A seven-atom linker was selected to provide optimum sensitivity of I_E/I_M to hydrophobic association. The secondary amide group used to connect the two chromophores serves both to facilitate the synthesis of α,ω-bispyrenes with different linkers and to increase solubility in polar solvents.[9] The alcohol functionality permits attachment to the 5′ end of an oligonucleotide and also increases the solubility of **3** in polar solvents.[10]

Chart 1



The synthesis of the bispyrenyl alcohol **3** is outlined in Scheme 1. Condensation of 1-pyrenecarboxaldehyde with methyl 4-nitrobutyrate gives methyl 4-nitro-5-(1′-pyrenyl)pent-4-enoate (**4**) in 63% yield. Attempts at direct reduction of **4** to the amino alcohol **7** using $LiAlH_4$, which was reported by Gaur et al.[11] for analogous phenyl- and naphthyl-substituted nitro esters, were unsuccessful, yielding a mixture of partially reduced products. However, we found that sequential reduction of **4** provided a successful route to **7**. Selective reduction of the double bond with $NaBH_4$ in the presence of silica gel using the method of Sinhababu and Borchardt[12] affords methyl 4-nitro-5-(1′-pyrenyl)valerate (**5**) in 49% yield. Reduction of **5** by diisobutylaluminum hydride (DIBAL-H) provides 4-nitro-5-(1′-pyrenyl)pentan-1-ol (**6**) in 68% yield. Finally, the nitro group is reduced by $NaBH_4$ in the

(1) Winnik, F. M. *Chem. Rev. (Washington, D.C.)* **1993**, *93*, 587.
(2) (a) Fages, F.; Bodenant, B.; Weil, T. *J. Org. Chem.* **1996**, *61*, 3956. (b) Takeshita, M.; Shinkai, S. *Chem. Lett.* **1994**, 125. (c) Kierzek, R.; Li, Y.; Turner, D. H.; Bevilacqua, P. C. *J. Am. Chem. Soc.* **1993**, *115*, 4985. (d) Bevilacqua, P. C.; Kierzek, R.; Johnson, K. A.; Turner, D. H. *Science* **1992**, *258*, 1355. (e) Aoki, I.; Harada, T.; Sakaki, T.; Kawahara, Y.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1992**, 1341. (f) Aoki, I.; Kawabata, H.; Nakashima, K.; Shinkai, S. *J. Chem. Soc., Chem. Commun.* **1991**, 1771. (g) Telser, J.; Cruickshank, K. A.; Morrison, L. E.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 6966.
(3) (a) Zachariasse, K. A.; Duveneck, G.; Kühnle, W.; Leinhos, U.; Reynders, P. In *Photochemical Processes in Organized Molecular Systems*; Honda, K., Ed.; Elsevier Science Publishers B.V.: Amsterdam, The Netherlands, 1991; p 83. (b) Zachariasse, K. A.; Kühnle, W. *Z. Phys. Chem. NF* **1976**, *101*, 267. (c) Kühnle, W.; Zachariasse, K. A. *J. Photochem.* **1976**, *5*, 165.
(4) Zachariasse, K. A.; Kühnle, W.; Weller, A. *Chem. Phys. Lett.* **1978**, *59*, 375.
(5) Winnik, M. A.; Redpath, A. E. C.; Paton, K.; Danhelka, J. *Polymer* **1984**, *25*, 91.
(6) (a) Lewis, F. D.; Wu, T.; Burch, E. L.; Bassani, D. M.; Yang, J.; Schneider, S.; Jäger, W.; Letsinger, R. L. *J. Am. Chem. Soc.* **1995**, *117*, 8785. (b) Letsinger, R. L.; Wu, T. *J. Am. Chem. Soc.* **1994**, *116*, 811. (c) Lewis, F. D.; Zhang, Y.; Letsinger, R. L. Unpublished results.
(7) Kitamura, M.; Nimura, A.; Yamana, K.; Shimidzu, T. *Nucleic Acids Res. Symp. Ser.* **1991**, *25*, 67.
(8) Korshun, V. A.; Pestov, N. B.; Birikh, K. R.; Berlin, Y. A. *Bioconjugate Chem.* **1992**, *3*, 559.
(9) For a recent example of the synthesis of amide-linked bispyrenes, see: Lou, J.; Hatton, T. A.; Laibinis, P. E. *Anal. Chem.* **1997**, *69*, 1262.
(10) The synthesis of oligonucleotide conjugates of **3** and their use as fluorescent oligonucleotide probes have been reported; see: Lewis, F. D.; Zhang, Y.; Letsinger, R. L. *J. Am. Chem. Soc.* **1997**, *119*, 5451−5452.
(11) Gaur, S. P.; Jain, P. C.; Anand, N. *Indian J. Chem.* **1982**, *21B*, 46.
(12) Sinhababu, A. K.; Borchardt, R. T. *Tetrahedron Lett.* **1983**, *24*, 227.

S0022-3263(97)01043-8 CCC: $14.00

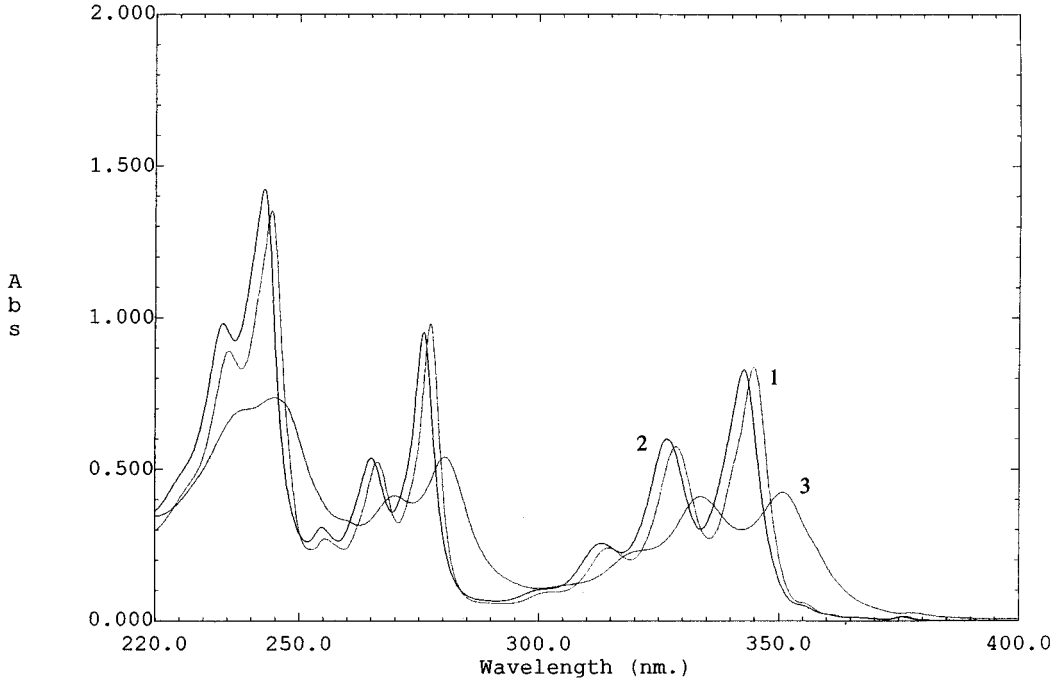
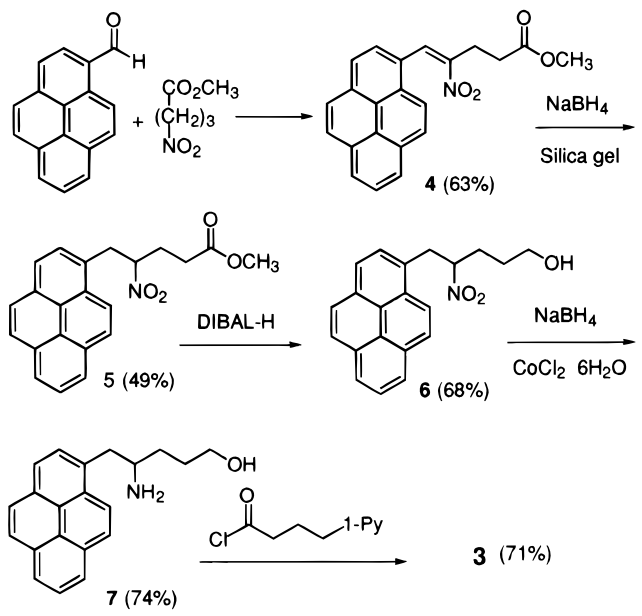


Figure 1. UV spectra of **3** (1×10^{-5} M) in different solvents: (1) THF, (2) CH_3OH, (3) H_2O.

Scheme 1



presence of $CoCl_2 \cdot 6H_2O$ to afford 4-amino-5-(1′-pyrenyl)-pentan-1-ol (**7**) in 71% yield. Reaction of **7** with 1-pyrenebutyryl chloride gives the bispyrenyl alcohol **3** in 71% yield.

The absorption spectra of **3** in THF, methanol, and water solutions (1×10^{-5} M) are shown in Figure 1. Values of P_A, the ratio of the absorption intensity for the lowest energy maxima and minima, are reported in Table 1. The value of P_A in water is substantially lower than the value in methanol or nonpolar solvents. The low value of P_A and the decrease in the extinction coefficient (hypochromism) in water are indicative of ground-state association of pyrene chromophores.[1,13] Chromophore association could result from either intra- or intermolecular association of pyrene chromophores. A plot of absorbance vs concentration for $(1 \times 10^{-6})-(1 \times 10^{-5})$ M aqueous solutions of **3** is linear. However at higher concentrations ($>10^{-5}$ M) time-dependent hypochromism is observed, indicative of aggregate formation.

The fluorescence spectra of **3** in THF, methanol, and methanol−water mixtures are shown in Figure 2. In THF and less polar solvents structured fluorescence typical of monomeric 1-substituted pyrenes is observed. The absence of excimer fluorescence is analogous to the results of Zachariasse and co-workers[3] for $Py(CH_2)_7Py$. It is interesting to note that a mixture of monomer and excimer fluorescence ($I_E/I_M \sim 0.5$) has been observed by Lou et al.[9] for an amide-linked bispyrene with a six-atom linker in a nonpolar solvent at 20 °C. Evidently the additional atom in the seven-atom linker of **3** is sufficient to make excimer formation thermodynamically unfavorable at room temperature in nonpolar solvents. The fluorescence spectrum of **3** in methanol solution displays a long-wavelength tail attributed to pyrene excimer. The ratio I_E/I_M, defined as the ratio of peak intensities at 462 and 376 nm, respectively, increases with water content in mixed methanol−water solutions and reaches a value of 17 in aqueous solution (Table 1). A similar increase in I_E/I_M in water vs methanol solution is observed for poly(ethylene oxide) end-labeled with pyrene,[5] and for pyrene-labeled (hydroxypropyl)cellulose. This increase has been attributed to hydrophobic association of pyrene in the ground state.[13]

The fluorescence decay times for monomer and excimer emission of **3** are reported in Table 1. Pyrene monomer emission from **3** in THF solution is dominated by a long-lived component, whose decay time is similar to that of 1-methylpyrene. As is the case for the dipyrenylalkanes, neither monomer nor excimer decay can be fit to a single exponential. Biexponential excimer decay from di(1-pyrenyl)alkanes has been attributed to emission from both fully overlapping (long-lived component) and partially overlapping (short-lived component) excimer ge-

(13) Winnik, F. M.; Tamai, N.; Yonezawa, Y.; Nishimura, Y.; Yamazaki, I. *J. Phys. Chem.* **1992**, *96*, 1967.

Table 1. P_A, I_E/I_M, and Fluorescence Lifetimes of Bispyrenyl Alcohol 3[a]

solvent	P_A[b]	I_E/I_M[c]	τ_M, ns (%)[d]	τ_E, ns (%)[e]
THF	3.09	0.12	191 (86), 82 (14)	
CH_3OH	2.74	0.43	181 (86), 64 (14)	186 (71), 106 (29)
CH_3OH/H_2O (1:1)		1.30	5 (61), 69 (27), 147 (22)	94 (83), 205 (17)
H_2O	1.41	17	*f*	22 (58), 66 (42)

[a] Data for nitrogen-purged solutions. [b] P_A is the ratio of I_{peak}/I_{valley} of the lowest energy absorption band in **3**. [c] I_E/I_M is the ratio of I_{462}/I_{376} from fluorescence spectra of **3**. [d] Decay times were measured at room temperature by single-photon counting with $\lambda_{em} = 376$ nm. Values in parentheses are the preexponentials for the decay components. [e] Decay times were measured at room temperature by single-photon counting with $\lambda_{em} = 530$ nm. Values in parentheses are the preexponentials for the decay components. [f] Monomer emission too weak for fluorescence decay measurement.

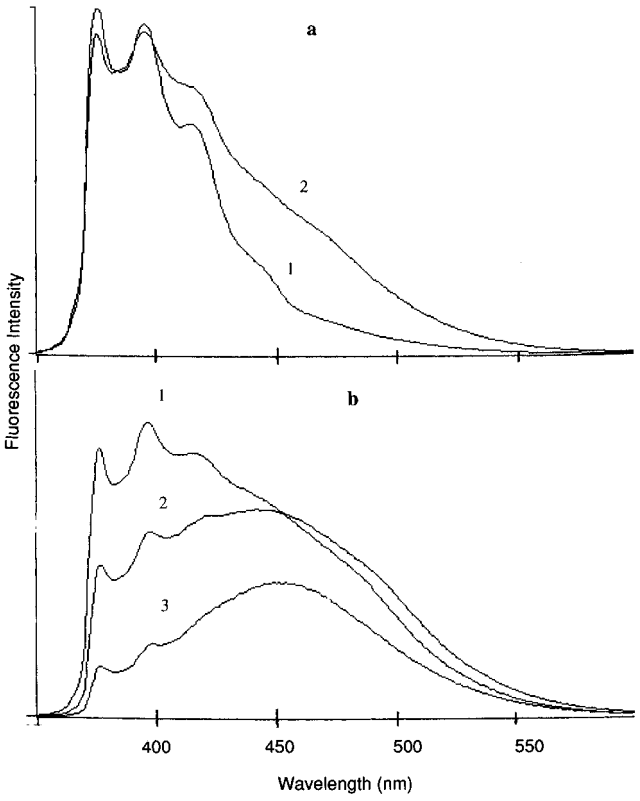


Figure 2. (a) Fluorescence spectra of **3** at room temperature in nitrogen-purged solution (1.67×10^{-6} M, $\lambda_{ex} = 335$ nm): (1) THF, (2) CH_3OH. (b) Fluorescence spectra of **3** at room temperature in nitrogen-purged solution (1.67×10^{-6} M, $\lambda_{ex} = 335$ nm): (1) CH_3OH/H_2O (3:1), (2) CH_3OH/H_2O (1:1), (3) CH_3OH/H_2O (1:2).

ometries.[14] The excimer fluorescence decay of **3** in aqueous solution does not display a rising component, which would be expected if excimer formation required a collisional encounter between singlet and ground-state chromophores in a bispyrene with an extended ground-state conformation. Ground-state pyrene association in aqueous solution is consistent with the absence of a rising component in the excimer fluorescence decay of **3**, as well as the broadening and hypochromism observed in the absorption spectrum (Figure 1).

The formation of ground-state pyrene dimers in **3** provides an interesting contrast to the failure of Wilcox[15] and Gellman[16] to observe intramolecular ground-state benzene or naphthalene dimers in solution. Theoretical evaluation of the pyrene ground-state dimer by Warshel and Huler[17] indicates that it adopts an offset face-to-face geometry and has a binding energy of 14 kcal/mol. This energy is too small to overcome the desolvation energy for intermolecular dimer formation but may be sufficient to permit intramolecular dimer formation in hydroxylic solvents. Formation of face-to-face dimers is more favorable for pyrene than for naphthalene or benzene both in solution and in the solid state.[18] Pyrene forms sandwich pairs in the crystal which display excimer fluorescence, whereas benzene and naphthalene adopt herringbone structures which display only perturbed monomer fluorescence.[19] The large variation of I_E/I_M for **3** in methanol−water-mixed solvent suggests that it may be a useful hydrophobic probe, both by itself and when covalently attached to oligonucleotides or polypeptides.

Experimental Section

General. NMR spectra were recorded at 300 Hz for proton frequency and 75 Hz for carbon frequency in $CDCl_3$ solution. The chemical shifts are reported in ppm (δ) relative to tetramethylsilane. Mass spectra were measured using electron impact ionization with an ionization voltage of 70 eV. Peaks are expressed as *m/z* (percent intensity relative to base peak). Melting points are uncorrected. All solvents and reagents were obtained from commercial sources and used without further purification, unless otherwise noted. Tetrahydrofuran (THF) and toluene were distilled from Na, while chloroform and triethylamine were distilled from CaH_2. Piperidine acetate[20] and 1-pyrenebutyryl chloride[21] were synthesized according to the literature procedures.

Methyl 4-Nitro-5-(1′-pyrenyl)pent-4-enoate (4). A mixture of 1-pyrenecarboxaldehyde (12.66 g, 55 mmol), methyl 4-nitrobutyrate (10.01 g, 66 mmol), and piperidine acetate (2.8 g, 19.4 mmol) in toluene (280 mL) was heated at reflux for 18 h. The organic layer was then washed with brine, H_2O, and dried ($MgSO_4$). Removal of the solvent gave a crude oil, and purification by column chromatography (silica gel, C_6H_6) afforded 12.8 g (63%) of reddish solid: mp 86−89 °C; ^{1}H NMR ($CDCl_3$) δ 2.68 (t, 2H, $J = 7.8$ Hz), 3.19 (t, 2H, $J = 7.8$ Hz), 3.54 (s, 3H), 7.90 (d, 1H, $J = 7.9$ Hz), 8.05−8.30 (m, 8H), 8.96 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.04, 32.03, 51.77, 123.06, 124.32, 124.66, 124.76, 125.82, 125.85, 126.16, 126.26, 126.50, 127.16, 128.84, 128.96, 129.68, 130.68, 131.17, 132.47, 134.22, 151.39, 172.03; MS (EI, 70 eV) *m/z* (relative intensity) 359 (M^+, 63), 312 (23), 239 (100), 202 (28), 157 (6), 126 (10), 113 (6).

Methyl 4-Nitro-5-(1′-pyrenyl)valerate (5). Under a N_2 atmosphere, $NaBH_4$ (2.85 g, 73 mmol) was added in three portions to a mixture of **4** (6.40 g, 17.8 mmol), silica gel (36 g), 2-propanol (55 mL), and chloroform (280 mL) over a period of 15 min at rt. The reaction was quenched by 5% HCl after 50 min, and the precipitate was filtered. The filtrate was then washed with CH_2Cl_2 and $CHCl_3$, and the combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated in vacuum. The crude product was subject to column chroma-

(14) De Schryver, F. C.; Collart, P.; Vandendriessche, J.; Goedeweeck, R.; Swinnen, A.; Van der Auweraer, M. *Acc. Chem. Res.* **1987**, *20*, 159.
(15) Paliwal, S.; Geib, S.; Wilcox, C. S. *J. Am. Chem. Soc.* **1994**, *116*, 4497.
(16) Newcomb, L. F.; Haque, T. S.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 6509.
(17) Warschel, A.; Huler, E. *Chem. Phys.* **1974**, *6*, 463.
(18) Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525.
(19) Stevens, B. *Spectrochem. Acta* **1962**, *18*, 439.
(20) Binovi, L. J.; Arlt, H. G., Jr. *J. Org. Chem.* **1961**, *26*, 1656.
(21) Aoki, I.; Kawahara, Y.; Sakaki, T.; Harada, T.; Shinkai, S. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 927.

tography (silica gel, C_6H_6) to afford 3.14 g (49%) of a light yellow solid: mp 93−96 °C; ^{1}H NMR ($CDCl_3$) δ 2.20−2.60 (m, 4H), 3.65 (s, 3H), 3.75−3.90 (m, 1H), 3.95−4.10 (m, 1H), 5.10 (m, 1H), 7.81 (d, 1H, J = 7.8 Hz), 7.95−8.35 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 28.44, 30.04, 37.73, 51.86, 88.55, 122.04, 124.76, 124.98, 125.12, 125.30, 125.54, 126.13, 127.36, 127.52, 127.85, 128.44, 128.62, 128.93, 130.65, 131.03, 131.29, 172.12; MS (EI, 70 eV) *m*/*z* (relative intensity) 361 (M^+, 76), 314 (63), 241 (84), 215 (100), 189 (10), 157 (4), 120 (8).

4-Nitro-5-(1′-pyrenyl)pentan-1-ol (6). Under a N_2 atmosphere, a solution of **5** (440 mg, 1.21 mmol) in dry toluene (5 mL) was added dropwise to a mixture of diisobutylaluminum hydride (DIBAL-H) (1.5 M in toluene, 3.0 mL, 4.5 mmol) and dry toluene (10 mL) at 0 °C. The mixture was reacted then for 20 min at that temperature and the reaction quenched by 5% HCl. The organic layer was separated, washed with NaCl, dried ($MgSO_4$), and concentrated in vacuum. The crude product was purified by column chromatography (silica gel, 10:1 C_6H_6/CH_3OH) to afford 273 mg (68%) of light yellow solid: mp 93−96 °C; ^{1}H NMR ($CDCl_3$) δ 1.30 (br s, 1H), 1.40−1.60 (m, 2H), 1.80−2.00 (m, 1H), 2.05−2.25 (m, 1H), 3.45−3.55 (m, 2H), 3.65−3.75 (m, 1H), 3.85−4.00 (m, 1H), 4.85−5.05 (m, 1H), 7.74 (d, 1H, J = 7.9 Hz), 7.85−8.25 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 28.77, 30.16, 37.86, 61.69, 89.66, 122.19, 124.78, 125.03, 125.11, 125.34, 125.59, 126.20, 127.44, 127.53, 127.96, 128.40, 128.92, 129.06, 130.67, 130.97, 131.32; MS (EI, 70 eV) *m*/*z* (relative intensity) 333 (M^+, 52), 286 (55), 241 (33), 227 (28), 215 (100), 202 (20), 189 (11), 120 (4).

4-Amino-5-(1′-pyrenyl)pentan-1-ol (7). A solution of **6** (1.0 g, 3 mmol) in CH_3OH (100 mL) was added slowly to $CoCl_2 \cdot 6H_2O$ (1.36 g, 5.71 mmol). Then, $NaBH_4$ (1.04 g, 27.3 mmol) was added to the above mixture in several portions. After 40 min, the reaction was quenched by 5% HCl, and the pH of the solution was adjusted to 3−4. The aqueous layer was then extracted by $CHCl_3$ and Et_2O for several times. After that, the aqueous layer was basified to pH > 10 and extracted by both $CHCl_3$ and Et_2O. The combined organic layers was dried ($MgSO_4$) and concentrated in vacuum to afford 670 mg (74%) of white solid: mp 128−130 °C; ^{1}H NMR ($CDCl_3$) δ 1.50−2.00 (m, 4H), 2.30−3.00 (br s, 1H), 3.15−3.30 (m, 2H), 3.50−3.70 (m, 3H), 7.82 (d, 1H, J = 7.8 Hz), 7.95−8.30 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 30.69, 36.37, 42.79, 53.29, 62.93, 123.27, 124.84, 124.94, 124.99, 125.21, 126.06, 127.06, 127.49, 127.65, 128.26, 129.23, 130.28, 130.85, 131.43, 133.32; FABMS *m*/*z* 303 (M^+). Anal. Calcd for $C_{21}H_{21}NO$: C, 83.13; H, 6.98; N, 4.62. Found: C, 82.73; H, 6.86; N, 4.59.

***N*-[4-(1′-Pyrenylmethyl)-1-hydroxylbutyl]-1′-pyrenebutyramide (3).** Under a N_2 atmosphere, a solution of 1-pyrenebutyryl chloride (600 mg, 1.95 mmol) in dry THF (10 mL) was added dropwise to a mixture of **7** (530 mg, 1.75 mmol), triethylamine (1 mL, 7.16 mmol), dry CH_3OH (60 mL), and dry THF (5 mL) at rt. The mixture was then stirred at rt for 24 h, and the solid was collected by filtration. After that, the solid was dissolved in CH_2Cl_2 (150 mL) and H_2O (50 mL). The organic layer was further washed with H_2O and dried ($MgSO_4$). Removal of the solvent afforded 718 mg (71%) of light yellow solid: mp 171−174 °C; ^{1}H NMR ($CDCl_3$) δ 1.20−1.70 (m, 4H), 1.95−2.20 (m, 5H), 3.10 (t, 2H, J = 7.7 Hz), 3.25−3.35 (m, 1H), 3.45−3.65 (m, 3H), 4.40−4.60 (m, 1H), 5.45 (d, 1H, J = 8.5 Hz), 7.60−8.20 (m, 17H), 8.40 (d, 1H, J = 9.2 Hz); ^{13}C NMR ($CDCl_3$) δ 27.32, 28.91, 30.84, 32.66, 36.31, 38.72, 50.71, 62.50, 123.31, 123.54, 124.63, 124.74, 124.81, 124.87, 124.92, 125.05, 125.88, 125.91, 126.71, 126.95, 127.27, 127.33, 127.51, 127.76, 128.39, 128.67, 129.89, 130.27, 130.80, 130.90, 131.31, 131.41, 132.08, 135.73, 172.75; HRMS calcd for $C_{41}H_{35}NO_2$ 573.267, found 573.266.

Acknowledgment. This research was supported by research grants from the National Institute of General Medical Sciences (GM 10265) and the National Science Foundation (CHE 9301381).

JO971043+