Fluorescent Heterocyclic Systems: Syntheses, Structures, and Physicochemical Properties of Dipyrido-Substituted 1,3,4,6-Tetraazapentalenes†

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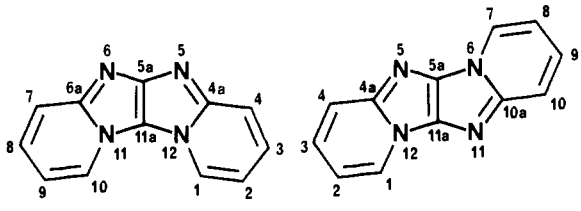
Abstract: The highly fluorescent dipyrido[1,2-*a*:1′,2′-*e*]-1,3,4,6-tetraazapentalene (**2**), anti isomer of the previously reported syn dipyrido-substituted 1,3,4,6-tetraazapentalene (**1**), has been prepared in two steps from 2-aminopyridine. The single-crystal X-ray structures of the two isomers are presented, and the physicochemical properties and spectral data of **1** and **2** are compared. Both tetraazapentalenes form dark-colored charge-transfer complexes with π-deficient aromatic compounds; the X-ray crystal structure of the complex of **1** with 2,4,7-trinitro-9-fluorenone has been determined. Both parent heterocycles and their *N*-ethyl bromide derivatives demonstrate an ability to illuminate the fragments of DNA in a manner similar to that with ethidium bromide. The stability of these two isomeric heterocycles, when considered with regard to the location of the four nitrogen atoms, is in accord with the tenets of topological charge stabilization theory.

Tetraazapentalenes are nitrogen-containing analogues of pentalene and, depending upon nitrogen arrangement and substitution, may be isoelectronic with the 10π-electron (Hückel aromatic) pentalene dianion.[1] Zwitterionic tetraazapentalenes possessing two junctional nitrogen atoms have been examined in some detail.[2] Although other non-zwitterionic tetraazapentalenes have been prepared,[3] only a few compounds based on a 10π-electron 1,3,4,6-tetraazapentalene nucleus are known: the photochemically prepared imidazo[4,5-*d*]imidazole reported by Ferris and Antonucci,[4] several thiazolo[3,2-*a*]-1,3,4,6-tetraazapentalenes,[5] and the fluorescent dipyrido[1,2-*a*:2′,1′-*f*]-1,3,4,6-tetraazapentalene (**1**) prepared in this Laboratory.[6] We now report the synthesis

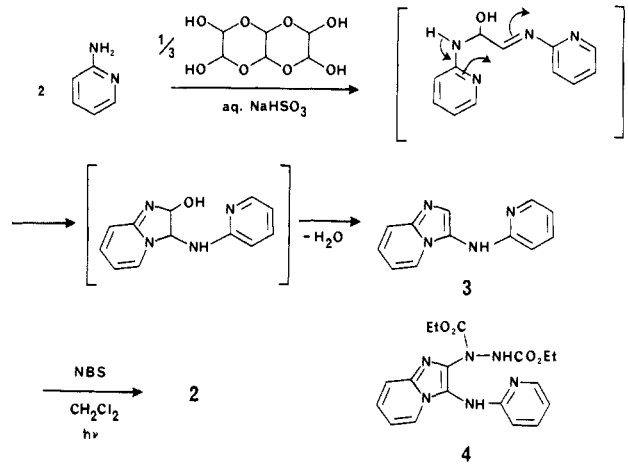


1 **2**

of the highly fluorescent anti dipyrido-substituted 1,3,4,6-tetraazapentalene **2**, along with a comparison of the structures and physicochemical properties of these two isomers, syn and anti in ring arrangement, and several of their derivatives.

The two-step synthesis of dipyrido[1,2-*a*:1′,2′-*e*]-1,3,4,6-tetraazapentalene (**2**) from 2-aminopyridine is outlined in Scheme I. Since the condensation of 2-aminopyridine with either formaldehyde bisulfite addition product[7a] or aliphatic aldehyde bisulfite addition products[7b] and cyanide ion is known to afford 3-aminoimidazo[1,2-*a*]pyridines, we reasoned that the condensation of 2-aminopyridine with 0.5 equiv of glyoxal would proceed by the initial formation of a bisimine intermediate (shown in its monohydrated form in Scheme I). This putative intermediate might then undergo an intramolecular annelation reaction and dehydration to give 3-(*N*-2-pyridinylamino)imidazo[1,2-*a*]pyridine (**3**). In practice, when a mixture of 2-aminopyridine and $^1/_6$ equiv of glyoxal trimer dihydrate was heated in aqueous $NaHSO_3$, the fluorescent compound **3** was obtained in 30% yield. The substitution of other amino-substituted heterocycles (2-aminopyrimidine, aminopyrazine, 3-aminopyridazine, 3-amino-1,2,4-triazine, amino-1,3,5-triazine, adenine, adenosine) for 2-aminopyridine in this procedure does not afford similarly annelated

Scheme I



compounds but instead proceeds with the formation of highly polar fluorescent products. In the cases of adenine and adenosine, the

† Presented in part: (a) Groziak, M. P.; Leonard, N. J. *Book of Abstracts*; 191st Meeting of the American Chemical Society, New York, New York, 1986; ORGN Division, Abstract 269. (b) Leonard, N. J. *Abstracts*; 10th International Congress of Heterocyclic Chemistry, Waterloo, Ontario, Canada; Aug 11-16, 1985; pp S6-33.

(1) For a review on aromatic azapentalenes, see: Elguero, J.; Claramunt, R. M., Summers, A. J. H. *Adv. Heterocycl. Chem.* **1978**, *22*, 183-320.

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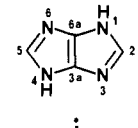
(4) (a) Ferris, J. P.; Antonucci, F. R. *J. Chem. Soc., Chem. Commun.* **1972**, 126-127. (b) Ferris, J. P.; Antonucci, F. R. *J. Am. Chem. Soc.* **1974**, *96*, 2010-2014. (c) Ferris, J. P.; Antonucci, F. R. *Ibid.* **1974**, *96*, 2014-2019. Imidazo[4,5-*d*]imidazole (i) is shown here in its most likely favored tautomeric form



i

(5) Dora, E. K.; Dash, B.; Panda, C. S. *J. Indian Chem. Soc.* **1979**, *56*, 620.

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Table I. Crystallographic and Intensity Data Collection Parameters for **1**, **2** and **1·TNF**[a]

	1	**2**	**1·TNF**
formula	$C_{12}H_8N_4 \cdot H_2O$	$C_{12}H_8N_4$	$C_{25}H_{13}N_7O_7$
formula wt, amu	226.24	208.22	523.42
crystal class	orthorhombic	monoclinic	monoclinic
space group	$Pbca(D_{2h}^{15})$	$P2_1/c(C_{2h}^5)$	$P2_1/n(C_{2h}^5)$
dimensns, mm	0.08 × 0.08 × 0.78	0.1 × 0.2 × 0.8	0.14 × 0.24 × 0.52
a, Å	18.729 (5)	6.812 (2)	12.407 (3)
b, Å	13.288 (2)	4.305 (1)	14.208 (3)
c, Å	8.112 (3)	16.547 (5)	13.392 (3)
β, deg	90	99.86 (2)	110.95 (2)
V, Å^3	2019 (1)	478.1 (3)	2204.6 (8)
Z	8	2	4
ρ (calcd), g/cm^3	1.488	1.446	1.577
radiatn, λ, Å	Mo Kα, 0.71073	Mo Kα, 0.71073	Cu Kα, 1.54178
octants, scan mode	$+h,-k,-l$, ω/θ	$\pm h,+k,+l$, $\omega/2\theta$	$\pm h,-k,-l$, $\omega/2\theta$
μ, cm^{-1}	0.94	0.86	9.66
2θ range, deg	2.0–55.0	3.0–53.0	3.0–130.0
intensities measd	2719	1226	4248
unique intensities	2318	1005	3748
obsd reflectns	831 ($I > 1.96\delta(I)$)	505 ($I > 2.58\delta(I)$)	2534 ($I > 2.58\delta(I)$)
R	0.068	0.081	0.046
R_w	0.065	0.088	0.057
no. variables	127	87	392
p	0.02	0.04	0.01

[a] **1**, Dipyrido[1,2-*a*:2′1′-*f*]-1,3,4,6-tetraazapentalene; **2**, dipyrido[1,2-*a*:1′,2′-*e*]-1,3,4,6-tetraazapentalene; **1·TNF**, dipyrido[1,2-*a*:2′,1′-*f*]-1,3,4,6-tetraazapentalene 2,4,7-trinitro-9-fluorenone complex.

products have previously been assigned structures consisting of an initially formed imine that apparently does not undergo further reaction.[8] Glyoxal is reported to react with *N*-alkylanilines to afford indole derivatives[9] and is known to afford labile 1:1 adducts with guanine and guanosine.[10] Thus, it appears that the utility of this annelation reaction may be limited to aromatic or heteroaromatic amines possessing a moderately nucleophilic ring atom ortho to the amine moiety.

The oxidative cyclization of **3** to **2** (32% yield) proceeds under photochemically generated free radical conditions (NBS in CH_2Cl_2, *hν*) but not under the ionic conditions (iodobenzene diacetate in CF_3CH_2OH) required for the preparation of the syn dipyrido-substituted isomer **1**.[6] The use of diethyl azodicarboxylate as an alternative oxidative cyclization reagent[11] likewise did not afford **2** but rather gave the C-2-substituted imidazo[1,2-*a*]-pyridin-3-amine derivative **4**.

Compounds **1** and **2** are yellow and fluorescent. The crystals have high melting points and yet are sublimable at reduced pressure (200–220 °C/1–2 mmHg). Both the UV and fluorescence spectra of **2** show maxima at about 40 nm longer wavelength than those of **1**; the quantum yield for the fluorescence of **1** (0.41) in absolute ethanol is greater than that of **2** (0.27) in the same solvent.[12] The solubilities of **1** and **2** differ markedly in that **1** shows limited solubility and only in highly polar, protic solvents (H_2O, CH_3OH, CH_3CH_2OH), whereas **2** is readily soluble in less polar organic solvents ($CHCl_3$, CH_2Cl_2) but is relatively insoluble in the polar protic solvents. Molecular mechanics calculations (MM2)[13] of the dipole moment of **1** (5.623 D) and **2** (0.005 D) offer an explanation of the solubility observations.

Table II. Selected Bond Lengths and Angles for **1**

bond	length, Å (esd)	atoms	angle, deg (esd)
C1–C2	1.336 (8)	N12–C1–C2	118.4 (5)
C2–C3	1.406 (9)	C1–C2–C3	121.2 (6)
C3–C4	1.349 (9)	C2–C3–C4	120.7 (6)
C4–C4a	1.400 (8)	C3–C4–C4a	121.0 (6)
C4a–N5	1.331 (7)	C4–C4a–N12	115.5 (5)
N5–C5a	1.359 (7)	C4a–N12–C1	123.2 (4)
C5a–C11a	1.364 (8)	N12–C4a–N5	113.5 (4)
C11a–N12	1.365 (7)	C4a–N5–C5a	103.5 (4)
N12–C4a	1.430 (7)	N5–C5a–C11a	112.0 (5)
N12–C1	1.368 (7)	C5a–C11a–N12	108.6 (5)
C5a–N6	1.371 (7)	C11a–N12–C4a	102.4 (4)
N6–C6a	1.335 (7)	C11a–C5a–N6	111.2 (5)
C6a–N11	1.427 (7)	C5a–N6–C6a	104.5 (4)
N11–C11a	1.363 (7)	N6–C6a–N11	111.8 (5)
C6a–C7	1.410 (8)	C6a–N11–C11a	104.1 (4)
C7–C8	1.351 (10)	N11–C11a–C5a	108.3 (5)
C8–C9	1.413 (10)	N11–C6a–C7	117.5 (5)
C9–C10	1.360 (9)	C6a–C7–C8	119.6 (6)
C10–N11	1.378 (7)	C7–C8–C9	121.4 (6)
		C8–C9–C10	120.9 (6)
		C9–C10–N11	118.3 (5)
		C10–N11–C6a	122.3 (4)

The ^{1}H NMR spectra of **1** and **2** are quite similar; each shows four signals: two doublet and two multiplet resonances. The ^{13}C NMR spectra of these two compounds are diagnostic, however, in that the spectrum of **1** shows seven discrete signals whereas that of the centrosymmetric **2** shows only six signals. A complete assignment of the ^{1}H and ^{13}C NMR spectra of **1** and **2** was accomplished as follows: (a) assignment of the low-field doublet resonance in the ^{1}H NMR spectrum to the proton attached to a carbon atom adjacent to a junctional nitrogen atom (H-1 in each case);[14] (b) assignment of the remaining ^{1}H NMR resonances by means of homonuclear decoupling experiments; (c) assignment of the CH resonances in the ^{13}C NMR spectra by two-dimensional short-range ^{1}H–^{13}C heteronuclear shift correlation experiments; and (d) assignment of the quaternary carbon resonances in the proton-coupled ^{13}C NMR spectra by an analysis of the magnitude of the long-range heteronuclear coupling. In the proton-coupled ^{13}C NMR spectrum of **1**, the signal at 153.4 ppm assigned to the C5a carbon atom shows no long-range ^{1}H-coupling, whereas those

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(10) (a) Nakaya, K.; Takenaka, O.; Horinshi, H.; Shibata, K. *Biochim. Biophys. Acta* **1968**, *161*, 23–31. (b) Yuki, H.; Sempuku, C.; Park, M.; Takiura, K. *Anal. Biochem.* **1972**, *46*, 123–128.

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(12) Compound **1** undergoes a photochemical reaction in acetonitrile solution under UV irradiation, whereas compound **2** is stable under these conditions: preliminary findings, Halpern, A. M., Northeastern University, and this laboratory.

(13) The W. Clark Still version (Model 1.3) of the Allinger MM2 molecular mechanics program was used for structural analysis.

(14) (a) Bochis, R. J. et al. *J. Med. Chem.* **1978**, *21*, 235–237. (b) Bochis, R. J.; Olen, L. E.; Fisher, M. H.; Reamer, R. A.; Wilks, G.; Taylor, J. E.; Olson, G. *Ibid.* **1981**, *24*, 1483–1487.

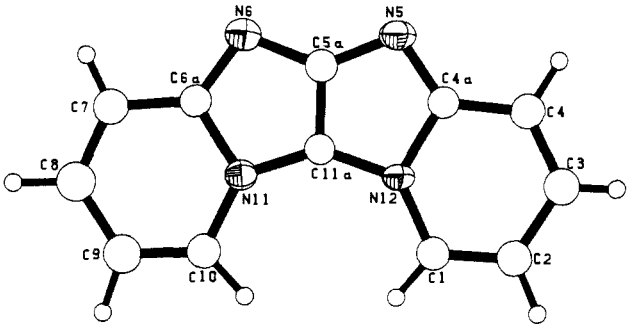

Figure 1. X-ray crystal structure of dipyrido[1,2-*a*:2′,1′-*f*]-1,3,4,6-tetraazapentalene (**1**).

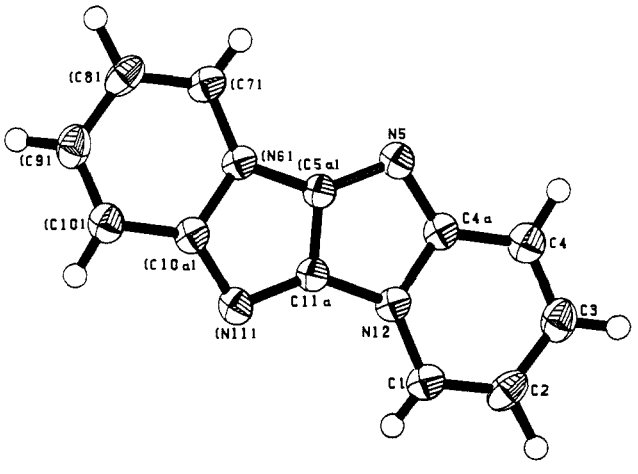

Figure 2. X-ray crystal structure of dipyrido[1,2-*a*:1′,2′-*e*]-1,3,4,6-tetraazapentalene (**2**).

at 116.7 ppm (C11a) and 148.4 ppm (C4a(6a)) show moderate and large long-range ^{1}H-coupling, respectively. Similarly, the signal at 134.6 ppm (C5a(11a)) in the proton-coupled ^{13}C NMR spectrum of **2** shows no long-range ^{1}H coupling, whereas that at 145.8 ppm (C4a(10a)) shows significant long-range coupling. The ^{13}C NMR chemical shift (134.6 ppm) of the junctional carbon atoms of the central imidazo[4,5-*d*]imidazole ring system in **2** is almost exactly equidistant from those in **1** (153.4 and 116.7 ppm), even though solubility limitations necessitated the use of a different deuterated solvent for **2**.

The single-crystal X-ray structure determinations of **1** and **2** reveal several interesting structural features (Tables I–III, Figures 1 and 2). Although the suitable crystal of **1** had been grown from "absolute" ethanol, the structure determination of **1** revealed a molecule of water (not shown in Figure 1) participating in a hydrogen bond with the lone-pair electrons on N6 and thus effecting a loss of symmetry. In the structure determination of **2**, half the atoms were located by a symmetry operation of reflection through the inversion center. The 6-5-5-6 fused ring system of each compound is planar to within 0.03 Å, and the carbon–carbon bonds in the periphery of the six-membered rings are of alternating length (1.336–1.360 (9) and 1.400–1.413 (10) Å), a finding indicative of the non-zwitterionic predominating resonance form. The internal bond angles of the imidazole ring at the N-5 and N-12 atoms of **2** (100.7 (4) and 104.4 (3) deg, respectively) are in accord with the pattern found for other imidazole derivatives, namely that the internal bond angle of the imino-type nitrogen atom is about 3–5 deg less than that of the trisubstituted nitrogen atom.[15] In contrast, the corresponding internal bond angles of **1**[6] present an exception to this pattern. Similar structural features were revealed by the MM2 molecular mechanics program.

Both **1** and **2** react readily with π-deficient planar aromatic compounds to form dark-colored, crystalline charge-transfer complexes. The complexes of **1** and **2** with 2,4,7-trinitro-9-fluorenone (TNF), 7,7,8,8-tetracyano-1,4-quinodimethane (TCQD), and 2,3-dichloro-5,6-dicyano-1,4-quinone (DDQ) were isolated and characterized. No complex was obtained from the reaction of **1** or **2** with trinitrobenzene or tetrathiafulvalene, probably because the former is an insufficiently reactive π-accepting species, and the latter is a π-donor. According to elemental microanalytical data, both tetraazapentalenes combined with the TNF and TCQD in a 1:1 ratio but combined with DDQ in a 3:2 ratio. The UV spectrum of each complex in methanolic solution appeared as the sum of the spectra of its components, indicating dissociation. Field desorption mass spectral analysis of each complex showed the molecular ion peaks (M^+) of each component. One of the isolated complexes, the dark-red **1·TNF**, was isolated in a crystalline form suitable for X-ray structural analysis; the results are shown in Table I and Figures 3 and 4. The structure determination of **1·TNF** showed a vertical stacking alternation of the two components of the complex. It is of interest that the least-squares planes of each component are not quite parallel (average separation distance of 3.3 Å, interplanar angle of 8.0°), and the orientation of the complex is such that the mono-nitrated

Table III. Selected Bond Lengths and Angles for **2**[a]

bond	length, Å (esd)	atoms	angle, deg (esd)
C1–C2	1.343 (8)	N12–C1–C2	118.8 (5)
C2–C3	1.405 (8)	C1–C2–C3	120.9 (5)
C3–C4	1.346 (8)	C2–C3–C4	120.5 (5)
C4–C4a	1.410 (7)	C3–C4–C4a	120.6 (5)
C4a–N5	1.352 (6)	C4–C4a–N12	116.5 (4)
N5–(C5a)	1.360 (6)	C4a–N12–C1	122.7 (4)
(C5a)–C11a	1.346 (6)	N12–C4a–N5	113.7 (4)
C11a–N12	1.389 (6)	C4a–N5–(C5a)	100.7 (4)
N12–C4a	1.406 (6)	N5–(C5a)–C11a	116.2 (4)
N12–C1	1.372 (6)	(C5a)–C11a–N12	105.0 (4)
		C11a–N12–C4a	104.4 (3)

[a] Atom C5a, shown in parentheses, is located by inversion of atom C11a through the center at (1/2,0,0).

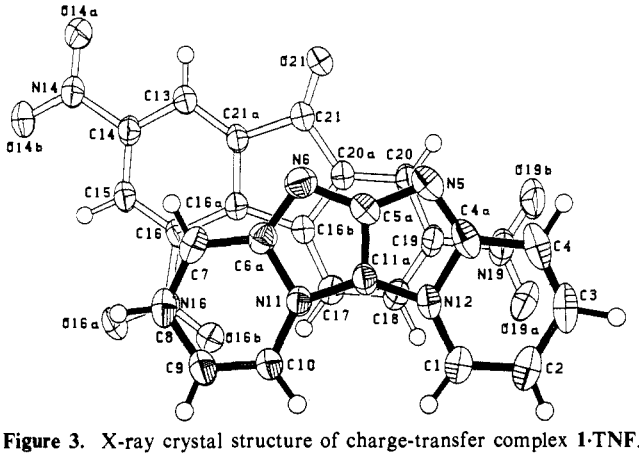

Figure 3. X-ray crystal structure of charge-transfer complex **1·TNF**.

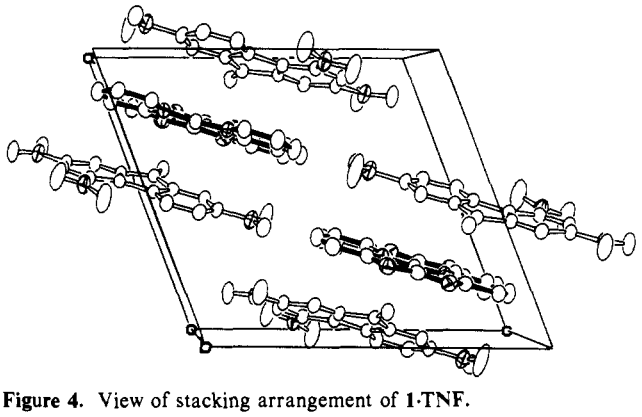

Figure 4. View of stacking arrangement of **1·TNF**.

(15) (a) Kálmán, A.; Argay, G. Y. *J. Mol. Struct.* **1983**, *102*, 391–402. (b) Speier, G.; Párkányi, L. *J. Org. Chem.* **1986**, *51*, 218–221.

ring of the TNF molecule resides over the central imidazo[4,5-*d*]imidazole ring system of **1**.

Both isomeric dipyrido-substituted tetraazapentalenes form crystalline picrate and *N*-ethyl bromide salts. Since the latter bear a structural resemblance to the fluorescent DNA-visualizing agent ethidium bromide,[16] we examined the ability of **1**, **1·EtBr**, **2**, and **2·EtBr** to permit visualization of a known mixture of DNA fragments and compared the results with those for ethidium bromide.[17-19] All four tetraazapentalenes exhibited DNA-visualizing behavior similar to that of ethidium bromide, albeit with less sensitivity. Ethidium bromide is known to undergo a significant fluorescence enhancement upon binding to oligonucleotides.[20]

The four nitrogen atoms in each of the two highly stable dipyrido-substituted 1,3,4,6-tetraazapentalenes **1** and **2** occupy sites of high charge density in their corresponding dianionic carbocyclic uniform reference frames, according to analysis by Hückel molecular orbital calculations. Thus, the large value of the sum of the charge densities of these sites for **1** and **2** (4.940 and 4.960, respectively) is in accord with the tenets of "topological charge stabilization" theory.[21]

Experimental Section

Dipyrido[1,2-*a*:2′,1′-*f*]-1,3,4,6-tetraazapentalene (Pyrido[1″,2″:1′,2′]-imidazo[4′,5′:4,5]imidazo[1,2-*a*]pyridine) (1). This compound was prepared according to our previously reported procedure:[6] ^{1}H NMR (300 MHz, CD_3OD) δ 8.85 (d, J = 6.8 Hz, 1, H-1 (10)), 7.62 (d, J = 9.3 Hz, 1, H-4 (7)), 7.43 (m, 1, H-3 (8)), 7.08 (m, 1, H-2 (9)); ^{13}C NMR (75.5 MHz, CD_3OD) δ 153.4 (C-5a), 148.4 (C-4a(6a)), 127.3 ($^1J_{CH}$ = 166 Hz, C-3 (8)), 126.0 ($^1J_{CH}$ = 187 Hz, C-1 (10)), 117.2 ($^1J_{CH}$ = 170 Hz, C-4 (7)), 116.7 (C-11a), 112.7 ($^1J_{CH}$ = 170 Hz, C-2 (9)); FT IR (KBr) 1630.4, 1519.3, 1512.7, 1506.1, 1487.5, 1399.7, 1331.5, 1231.7, 1222.2, 1193.3, 743.2, 734.8, 715.3 cm^{-1}; fluorescence λ^{em}_{max} 389 nm, λ^{ex}_{max} 355 nm, Φ = 0.41 (deoxygenated absolute ethanol) (relative to coumarin in deoxygenated absolute ethanol, Φ = 0.51 at λ^{ex} = 350 nm (measured relative to the reported value of Φ = 0.64 at λ^{ex} = 366 nm[22])) (all excitations at 350 nm).[23]

Picrate. A solution of **1** (21 mg, 0.1 mmol) in 5 mL of acetonitrile was treated with a solution of 2,4,6-trinitrophenol (23 mg, 0.1 mmol) in 2 mL of acetonitrile, and the mixture was heated on a steam bath. The mixture was allowed to cool to room temperature, and the product was isolated by suction filtration, washed with a small amount of diethyl ether, and pumped dry at room temperature to afford 25 mg (57%) of product as a yellow solid: mp 280–281 °C (dec); ^{1}H NMR (360 MHz, $(CD_3)_2SO$) δ 9.49 (d, J = 6.6 Hz, 2, ArH), 8.56 (s, 2, ArH), 8.01 (d, J = 9.1 Hz, 2, ArH), 7.53 (m, 2, ArH); IR (KBr) 3084, 1627, 1608, 1546 cm^{-1}; UV λ_{max}, nm ($\epsilon \times 10^4$) (CH_3OH) 255 (2.2), 262 (2.2), 286 (0.9), 299 (0.9); FD MS, m/z 208 (M^+), 229 (M^+) amu. Anal. Calcd for $C_{18}H_{11}N_7O_7$: C, 49.44; H, 2.54; N, 22.42. Found: C, 49.28; H, 2.42; N, 22.05.

***N*-Ethyl Bromide Salt.** A mixture of **1** (104 mg, 0.5 mmol) and bromoethane (164 μL, 2.2 mmol) in 1.0 mL of anhydrous *N,N*-dimethylacetamide under nitrogen was heated at 150 °C for 2 h and then was allowed to cool to room temperature. The mixture was diluted with 10 mL of anhydrous diethyl ether, and the product was collected by suction filtration, washed with a small amount of diethyl ether, and then recrystallized from methanol/diethyl ether to afford 132 mg (83%) of product as a pale yellow powder: mp 281–283 °C; ^{1}H NMR (360 MHz, $(CD_3)_2SO$) δ 9.67 (d, J = 6.4 Hz, 1, ArH), 9.46 (d, J = 6.4 Hz, 1, ArH), 8.51 (d, J = 9.1 Hz, 1, ArH), 8.17 (m, 1, ArH), 8.00 (d, J = 9.1 Hz, 1, ArH), 7.77 (m, 1, ArH), 7.42 (m, 2, ArH), 4.75 (q, J = 7.1 Hz, 2, CH_2), 1.62 (t, J = 7.1 Hz, 3, CH_3); ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$) δ 147.8, 142.1, 139.2, 132.5, 128.2, 127.5, 117.6, 117.2, 115.2, 113.0, 111.5, 40.0, 16.9; IR (KBr) 2895, 2360, 2334, 1506, 1467 cm^{-1}; UV λ_{max}, nm ($\epsilon \times 10^4$) (CH_3OH) 233 (3.1), 243 (2.5), 278 (1.2), 287 (1.2), 334 (2.0); FD MS, m/z 237 ($M - Br^-$) amu. An analytical sample was recrystallized from ethanol/ethyl acetate. Anal. Calcd for $C_{14}H_{13}BrN_4$: C, 53.01; H, 4.13; Br, 25.19; N, 17.66. Found: C, 52.75; H, 4.13; Br, 25.48; N, 17.40.

3-(*N*-2-Pyridinylamino)imidazo[1,2-*a*]pyridine (*N*-(2-Pyridinyl)-imidazo[1,2-*a*]pyridin-3-amine) (3). A mixture of glyoxal trimer dihydrate (26.3 g, 0.375 mol) and $Na_2S_2O_5$ (85.5 g, 0.9 mol) in 300 mL of N_2-degassed water was heated on a steam bath until homogeneous (15 min), then treated with 2-aminopyridine (70.6 g, 0.75 mol), and heated for an additional 12 h. The reaction mixture was allowed to cool to room temperature with vigorous stirring, and the resulting precipitate was collected by suction filtration and washed with a small amount of water to afford, after drying in vacuo at 100 °C, 22.2 g (28%) of **3** as a slightly fluorescent, white powder. Additional **3** (1.74 g, total: 30%) was obtained by extracting the combined aqueous layers with chloroform. Recrystallization from acetone afforded pure **3**: mp 175–177 °C; ^{1}H NMR (360 MHz, $(CD_3)_2SO$) δ 8.81 (s, exchanges with D_2O, 1, NH), 7.99 (m, 1, ArH), 7.93 (m, 1, ArH), 7.54 (m, 2, ArH), 7.49 (s, 1, H-2), 7.23 (m, 1, ArH), 6.88 (m, 1, ArH), 6.72 (m, 1, ArH), 6.62 (d, J = 8.4 Hz, 1, ArH); IR (KBr) 2903, 1600, 1499, 1477, 1134 cm^{-1} UV λ_{max} ($\epsilon \times 10^4$) (CH_3OH) 210 (2.0), 231 (2.3), 280 (0.7), 291 (0.7); (H_2O) (pH 1) 206 (1.5), 224 (1.0), 282 (0.8); (pH 11) 230 (2.2), 290 (0.8); EI MS, (70 eV) m/z (rel intensity) 210 (M^+, 100), 132 ($M^+ - C_5H_4N$, 27), 105 ($M^+ - C_6H_5N_2$, 38) amu. Anal. Calcd for $C_{12}H_{10}N_4$: C, 68.56; H, 4.79; N, 26.65. Found: C, 68.87; H, 4.86; N, 26.64.

2-[1,2-Bis(ethoxycarbonyl)hydrazino]-3-(*N*-2-pyridinylamino)-imidazo[1,2-*a*]pyridine (4). A solution of **3** (4.25 g, 20 mmol) in 60 mL of anhydrous *N,N*-dimethylacetamide under nitrogen was cooled to 0 °C and treated dropwise with diethyl azodicarboxylate (4 mL, 25.4 mmol). The reaction mixture was stirred at room temperature for 30 min and then rotary evaporated in vacuo below 45 °C. The residue was triturated with 50 mL of acetone, and the product was collected by suction filtration, washed with a small amount of acetone, and pumped dry in vacuo at room temperature to afford 4.13 g (53%) of **4** as a pale yellow powder. Recrystallization from acetone gave pure **4**: mp 220–221 °C; ^{1}H NMR (360 MHz, $(CD_3)_2SO$) δ 10.02 (s, exchanges with D_2O, 1, NH), 7.96 (d, J = 4.1 Hz, 1, ArH), 7.31 (m, 1, ArH), 6.93 (m, 1, ArH), 6.75 (m, 1, ArH), 6.58 (d, J = 8.3 Hz, 1, ArH), 4.01 (q, J = 7.0 Hz, 4, 2-OCH_2), 1.12 (t, J = 7.0 Hz, 6, 2-CH_3); IR (KBr) 3300, 3122, 2891, 1735, 1701, 1477, 1252 cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^4$) (CH_3OH) 229 (2.6), 289 (0.8); (H_2O) (pH 1) 213 (3.0), 283 (1.4); (pH 11) 215 (2.7), 233 (2.6), 290 (0.8); FAB MS, m/z 285 (MH^+) amu. Anal. Calcd for $C_{18}H_{20}N_6O_4$: C, 56.24; H, 5.24; N, 21.86. Found: C, 56.00; H, 5.21; N, 21.73.

Dipyrido[1,2-*a*:1′2′-*e*]-1,3,4,6-tetraazapentalene (Pyrido[2″,1″:2′,3′]-imidazo[4′,5′:4,5]imidazo[1,2-*a*]pyridine) (2). A solution of **3** (8.0 g, 32 mmol) in 250 mL of dry dichloromethane under nitrogen was irradiated with a 150-W sunlamp at reflux and was then treated dropwise with a solution of *N*-bromosuccinimide (7.1 g, 40 mmol) in 250 mL of dry dichloromethane over 30 min. The dark reaction mixture was stirred under irradiation for 1 h, allowed to cool to room temperature, and then treated with 200 mL of saturated aqueous K_2CO_3. The layers were separated, and the organic phase was washed with saturated aqueous K_2CO_3 (1 × 200 mL), water (2 × 200 mL), and brine (1 × 100 mL), dried (Na_2SO_4), and rotary evaporated onto 50 g of silica gel. This was applied to a chromatography column prepacked with 250 g of silica gel in 10% methanol/chloroform. Elution of the column with this same solvent system afforded 2.5 g (32%) of **2** as a pale yellow solid: mp 292–294 °C (dec) (from Me_2CO); ^{1}H NMR (360 MHz, $(CD_3)_2SO$) δ 8.79 (d, J = 7.1 Hz, 1, H-1 (7)), 7.75 (d, J = 9.3 Hz, 1, H-4) (10)), 7.42 (m, 1, H-3 (9)), 7.07 (m, 1, H-2 (8)); ^{13}C NMR (90.4 MHz, $(CD_3)_2SO$) δ 145.8 (C-4a (10a)), 134.6 (C-5a (11a)), 124.9 ($^1J_{CH}$ = 164 Hz, C-3 (9)), 123.9 ($^1J_{CH}$ = 185 Hz, C-1 (7)), 118.0 ($^1J_{CH}$ = 167 Hz, C-4 (10)), 111.1 ($^1J_{CH}$ = 167 Hz, C-2 (8)); FT IR (KBr) 1621.4, 1529.7, 1503.2, 1464.1, 1422.1, 1334.9, 1302.1, 1251.0, 1186.9, 1136.7, 1127.6, 1100.7, 740.8, 723.0, 640.4, 440.1 cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^4$): (CH_3OH) 221 (2.1), 258 (4.1), 358 (1.3), 375 (1.5), 396 (1.0); (H_2O) (pH 1) 215 (1.6), 252 (3.2), 354 (1.5); (pH 11) 219 (1.4), 256 (4.0), 356 (1.4), 372 (1.6), 392 (1.0); fluorescence λ^{em}_{max} 433 nm, λ^{ex}_{max} 393 nm, Φ = 0.27 (deoxygenated absolute ethanol) (relative to coumarin in deoxygenated absolute ethanol, Φ = 0.51 at λ^{ex} = 350 nm (measured relative to the reported value of Φ = 0.64 at λ^{ex} = 366 nm[22])) (all excitations at 350 nm); low-resolution FAB MS, m/z (rel intensity) 209 (MH^+, 83), 155 (47), 135 (84), 119 (100), 103 (64); High-resolution FAB MS, m/z 209.0820 ($C_{12}H_9N_4$ (MH^+) requires 209.0827) amu. Anal. Calcd for $C_{12}H_8N_4$: C, 69.22; H, 3.87; N, 26.91. Found: C, 69.33; H, 3.67; N, 27.02.

Picrate: (35%) mp 278–281 °C (dec); ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 8.82 (d, J = 9 Hz, 2, ArH), 8.59 (s, 2, ArH), 7.83 (d, J = 9 Hz, 2, ArH), 7.52 (m, 2, ArH), 7.17 (m, 2, ArH); IR (KBr) 1613, 1571, 1356, 1315, 1269 cm^{-1}. UV λ_{max} nm ($\epsilon \times 10^4$) (CH_3OH) 256

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(2.0), 343 (1.0), 357 (1.0), 357 (1.0), 373 (1.0), 395 (0.7); FD MS m/z 208 (M^+) and 229 (M^+) amu. Anal. Calcd for $C_{18}H_{11}N_7O_7$: C, 49.44; H, 2.54; N, 22.42. Found: C, 49.35; H, 2.50; N, 22.17.

***N*-Ethyl Bromide Salt**: (97%) mp 262–263 °C; ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 9.33 (d, J = 7 Hz, 1, ArH), 9.11 (d, J = 7 Hz, 1, ArH), 8.56 (d, J = 9 Hz, 1, ArH), 8.22 (m, 1, ArH), 7.96 (d, J = 9 Hz, 1, ArH), 7.73 (m, 1, ArH), 7.66 (m, 1, ArH), 7.33 (m, 1, ArH), 5.08 (q, J = 7 Hz, 2, CH_2), 1.56 (t, J = 7 Hz, 3, CH_3); IR (KBr) 3456, 2980, 1631, 1514, 740 cm^{-1}; UV λ_{max} nm ($\epsilon \times 10^4$) (CH_3OH) 254 (3.4), 362 (1.3); FD MS, m/z 237 ($M - Br^-$) amu. An analytical sample was recrystallized from ethanol/ethyl acetate. Anal. Calcd for $C_{14}H_{13}BrN_4 \cdot H_2O$: C, 50.17; H, 4.51; N, 16.71. Found: C, 49.89; H, 4.26; N, 16.83.

Charge-Transfer Complexes of 1 and 2 with TNF, DDQ, and TCQD. 1·TNF. A solution of **1** (42 mg, 0.2 mmol) in 10 mL of dry acetonitrile was treated with a solution of 2,4,7-trinitro-9-fluorenone (63 mg, 0.2 mmol) in 2 mL of dry acetonitrile. The mixture was heated at reflux briefly on a steam bath, filtered while hot, and allowed to cool to room temperature. The product was collected by suction filtration, washed with a small amount of acetonitrile, and pumped dry at room temperature to afford 40 mg (38%) of a 1:1 complex as reddish-black needles: mp 243–245 °C; IR (KBr) 1725, 1596, 1522, 1511 cm^{-1}. FD MS m/z 208 (M^+) and 315 (M^+) amu. Anal. Calcd for $C_{25}H_{13}N_7O_7$: C, 57.37; H, 2.50; N, 18.73. Found: C, 57.22; H, 2.44; N, 18.47.

1·DDQ: (43%) tan powder; mp 264–268 °C; IR (KBr) 3078, 2218, 1735, 1550, 1473 cm^{-1}. FD MS, m/z 208 (M^+) and 227 (M^+) amu. Anal. Calcd for $C_{52}H_{24}N_{16}Cl_4O_4 \cdot 2H_2O$: C, 56.03; H, 2.53; N, 20.10. Found: C, 56.01; H, 2.43; N, 19.94.

1·TCQD: (66%) green-black powder; mp 280–285 °C; IR (KBr) 3032, 2218, 1627, 1532, 1521 cm^{-1}; FD MS, m/z 208 (M^+) and 204 (M^+) amu. Anal. Calcd for $C_{24}H_{12}N_8$: C, 69.90; H, 2.93; N, 27.17. Found: C, 69.56; H, 3.08; N, 27.01.

2·TNF: (71%) black needles; mp 240–242 °C (dec); IR (KBr) 1740, 1526, 1342, 750 cm^{-1}. FD MS, m/z 208 (M^+) and 315 (M^+) amu. Anal. Calcd for $C_{25}H_{13}N_7O_7$: C, 57.37; H, 2.50; N, 18.73. Found: C, 57.14; H, 2.44; N, 18.51.

2·DDQ: (38%) tan powder; mp >300 °C; IR (KBr) 1618, 1464, 1172, 1100, 783 cm^{-1}. FD MS, m/z: 208 (M^+) and 227 (M^+) amu. Anal. Calcd for $C_{58}H_{24}N_{16}Cl_4O_4$: C, 57.90; H, 2.19; N, 21.12. Found: C, 57.89; H, 2.24; N, 20.98.

2·TCQD: (61%) green-black powder; mp 290–293 °C (dec); IR (KBr) 2220, 1540, 1529, 1505, 1340 cm^{-1}. FD MS m/z 208 (M^+) and 204 (M^+) amu. Anal. Calcd for $C_{24}H_{12}N_8$: C, 69.90; H, 2.93; N, 27.17. Found: C, 69.51; H, 2.99; N, 27.02.

Comparison of DNA-Illuminating Property of 1, 1·EtBr, 2, 2·EtBr, and Ethidium Bromide.[18] An agarose gel consisting of 0.3 g of agarose in 30 mL of 1 × TBE (90 mM Tris, 65 mM boric acid, and 2.5 mM EDTA, disodium salt) was prepared at 60 °C and poured onto a minigel glass plate equipped with combs. A sample of λHind III-digested DNA was prepared from 1 μL of 0.52 ng/μL DNA, 4 μL of 1 × TBE, and 1 μL of dye, heated for 2 min at 68 °C, and then loaded onto the agarose gel in five successive lanes. The gel was then subjected to electrophoresis at 75 V for 1 h (until the bromthymol blue dye had migrated $^2/_3$ the distance of the gel). The lanes were separated and stained for 45 min in 30 mL of a 2.40 μM aqueous solution of each of the following heterocycles: ethidium bromide (Sigma), **1**, **1·EtBr**, **2**, and **2·EtBr**. The gels were then destained for 60 min in deionized, distilled water and photographed under shortwave ultraviolet illumination with a Polaroid camera equipped with a red filter.

Single-Crystal X-ray Structure Determination of 1, 2, and 1·TNF. Suitably sized crystals were mounted with epoxy to a thin glass fiber on either an Enraf-Nonius CAD 4 automated κ-axis or Syntex $P2_1$ automated four-circle diffractometer. Graphite monochromated radiation was used for the data collected for each of the three compounds as described in Table I. All space groups were determined unambiguously by systematic conditions, and all structures were solved by direct methods by using either MULTAN 80 or SHELX 76 programs. Subsequent least-squares difference Fourier calculations in each case revealed the positions for all of the hydrogen atoms. For each final cycle of least squares, all atomic positional parameters were refined independently. Successful convergence was indicated in all cases by the maximum shift/error for the last cycle. The final difference Fourier had no significant features.

Acknowledgment. This work was supported by the National Science Foundation (NSF CHE 84-16336) and in part by an unrestricted grant from Eli Lilly and Company. We thank Patti Silver for her assistance in the preparation of this manuscript.

Registry No. 1, 100460-12-6; **1**·picrate, 104716-53-2; **1**·EtBr, 104716-54-3; **1**·TNF, 104716-57-6; **1**·DDQ, 104716-58-7; **1**·TCQD, 104716-59-8; **2**, 104716-50-9; **2**·picrate, 104716-55-4; **2**·EtBr, 104716-56-5; **2**·TNF, 104716-60-1; **2**·DDQ, 104716-61-2; **2**·TCQD, 104716-62-3; **3**, 104716-51-0; **4**, 104716-52-1; glyoxal trimer dihydrate, 4405-13-4; 2-amino pyridine, 504-29-0; diethyl azodicarboxylate, 1972-28-7; ethyl bromide, 74-96-4.

Supplementary Material Available: Selected bond lengths and angles for **1**·TNF, thermal parameters, and atom coordinates for **1**, **2**, and **1**·TNF, least-squares planes, comparison of DNA-visualizing ability, 2D ^{1}H–^{13}C heteronuclear magnetic resonance shift correlation spectra for **1** and **2**, and general experimental data (17 pages). Ordering information is given on any current masthead page.