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Synthesis and Physical Studies of Azamitosene and Iminoazamitosene Reductive Alkylating Agents. Iminoquinone Hydrolytic Stability, Syn/Anti Isomerization, and Electrochemistry

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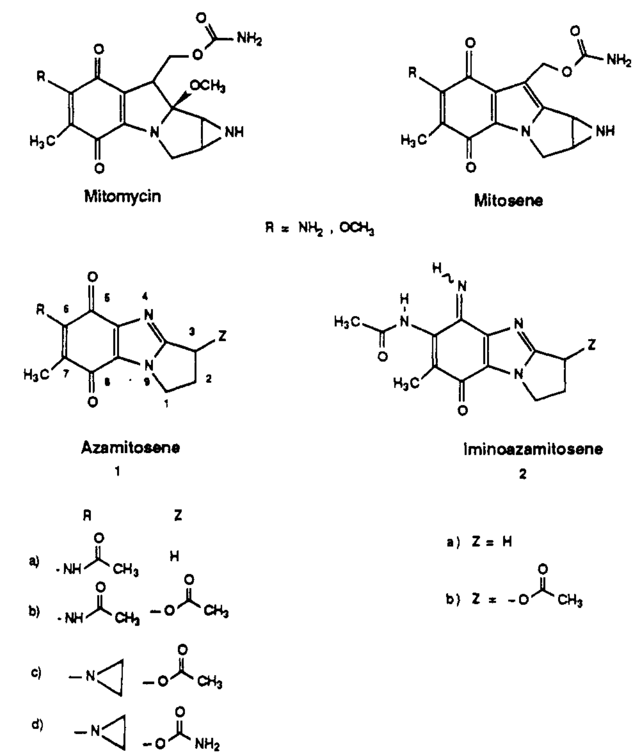
The synthesis of 2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-diones (azamitosenes) was carried out in conjunction with the design of potential DNA cross-linkers activated by reduction (reductive alkylation). These quinones resemble mitosene antitumor agents, but are based on the benzimidazole nucleus rather than the indole nucleus. Preliminary results indicate the azamitosenes are potent antitumor agents. Iminoquinone derivatives of azamitosenes (iminoazamitosenes) were synthesized as reductive alkylating agents exhibiting low oxygen toxicity. The iminoazamitosenes are hydrolytically stable in neutral buffers and undergo buffer-catalyzed syn/anti isomerization at the imino center. Electrochemical and oxygen reactivity studies in aqueous buffers indicate the change from quinone to iminoquinone is accompanied by an increase in reduction potential and a decrease in oxygen reactivity of the corresponding reduced species. It is concluded that iminoazamitosenes, and perhaps other iminoquinones, would exhibit low oxygen toxicity during cellular reductive alkylation.

Mitomycins and the corresponding mitosene analogues are well-known examples of reductive alkylating quinones.[2] The reductive alkylation process involves the formation of an alkylating quinone methide species upon reduction of the quinone and elimination of a leaving group.[3] Since tumor cells possess a low reduction potential environment,[4] there is a great deal of intrest in reductive alkylating quinones as selective antitumor agents. Thus a wide range of mitomycin and mitosene derivatives have been prepared in an effort to optimize antitumor activity.[5] All of these derivatives possess the indole ring nucleus, but with a variety of substituents.

Chart I



Efforts in this laboratory showed that benzimidazole-based reductive alkylating agents[6] are also capable of forming an alkylating quinone methide species. Altering the indole nucleus of mitosene to benzimidazole (azamitosene) therefore becomes important in terms of antitumor agent development.

A problem with reductive alkylating agents is the formation of toxic oxygen species by cycling between the quinone and hydroquinone forms of the agent.[7] In the case of daunomycin, the iminoquinone derivative of this reductive alkylating agent possesses lower oxygen toxicity than the quinone derivative.[8] Thus, another endeavor was

(1) National Institutes of Health Research Career Development Award Recipient (CA01349), 1988–1993.

(2) (a) Schwartz, H. S.; Sodergren, J. E.; Phillips, F. S. *Science (Washington, D.C.)* **1963**, *142*, 1181. (b) Iyer, V. N.; Szybalski, W. *Science (Washington, D.C.)* **1964**, *145*, 55. (c) Kennedy, K. A.; Rockwell, S.; Sartorelli, A. C. *Proc. Am. Assoc. Cancer Res.* **1979**, *20*, no. 1129, 278. (d) Kennedy, K. A.; Rockwell, S.; Sartorelli, A. C. *Cancer Res.* **1980**, *40*, 2356. (e) Tomasz, M.; Lipman, R.; Snyder, J. K.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 2059. (f) Tomasz, M.; Lipman, R.; Verdine, G. L.; Nakanishi, K. *Biochemistry* **1986**, *25*, 4337. (g) Tomasz, M.; Lipman, R.; McGuinness, B. F.; Nakanishi, K. *J. Am. Chem. Soc.* **1988**, *110*, 5892. (h) Tomasz, M.; Chawla, A. K.; Lipman, R. *Biochemistry* **1988**, *27*, 3182. (i) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science (Washington, D.C.)* **1987**, *235*, 1204. (j) Peterson, D. M.; Fisher, J. *Biochemistry* **1986**, *25*, 4077. (k) Andrews, P. A.; Pan, S.; Bachur, N. R. *J. Am. Chem. Soc.* **1986**, *108*, 4158. (l) Hornemann, U.; Keller, P. J.; Kozlowski, J. F. *J. Am. Chem. Soc.* **1979**, *101*, 7121. (m) Hornemann, U.; Iguchi, K.; Keller, P. J.; Vu, H. M.; Kozlowski, J. R.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5026. (n) Bean, M.; Kohn, H. *J. Org. Chem.* **1983**, *48*, 5033; *J. Org. Chem.* **1985**, *50*, 293. (o) Kohn, H.; Zein, N. *J. Am. Chem. Soc.* **1983**, *105*, 4105. (p) Zein, N.; Kohn, H. *Ibid.* **1986**, *108*, 296. (q) Kohn, H.; Zein, N.; Lin, X. Q.; Ding, J.-Q.; Kadish, K. M. *J. Am. Chem. Soc.* **1987**, *109*, 1833.

(3) (a) Moore, H. W. *Science (Washington, D.C.)* **1977**, *197*, 527. (b) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249.

(4) (a) Kennedy, K. A.; Teicher, B. A.; Rockwell, S.; Sartorelli, A. C. *Biochem. Pharm.* **1980**, *29*, 1. (b) Lin, A. J.; Sartorelli, A. C. *Biochem. Pharm.* **1976**, *25*, 206. (c) Kennedy, K. A.; Sligar, S. G.; Polomski, L.; Sartorelli, A. C. *Biochem. Pharmacol.* **1982**, *31*, 2011. (d) Kennedy, K. A.; Rockwell, S.; Sartorelli, A. C. *Cancer Res.* **1980**, *40*, 2356. (e) Keyes, S. R.; Heimbrook, D. C.; Fracasso, P. M.; Rockwell, S.; Sligar, S. G.; Sartorelli, A. C. *Adv. Enz. Reg.* **1985**, *23*, 291.

(5) For a review see: Remers, W. A. *The Chemistry of Antitumor Antibiotics*; Wiley-Interscience: New York, 1979; Vol. 1. (a) Iyengar, B. S.; Sami, S. M.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1983**, *26*, 16. (b) Sami, S. M.; Iyengar, B. S.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1984**, *27*, 701. (c) Vyas, D. M.; Chiang, Y.; Benigni, D.; Doyle, T. W. *J. Org. Chem.* **1987**, *52*, 5601. (d) Iyengar, B. S.; Dorr, R. T.; Remers, W. A.; Kowal, C. D. *J. Med. Chem.* **1988**, *31*, 1579. (e) Fishbein, P. L.; Kohn, H. *J. Med. Chem.* **1987**, *30*, 1767. (f) Sawhney, K. N.; Kohn, H. *J. Med. Chem.* **1989**, *32*, 248.

(6) Skibo, E. B. *J. Org. Chem.* **1986**, *51*, 522.

(7) (a) Doroshow, J. H. *Cancer Res.* **1983**, *43*, 460. (b) Begleiter, A. *Cancer Res.* **1983**, *43*, 481.

(8) Tong, G. L.; Henry, D. W.; Acton, E. M. *J. Med. Chem.* **1979**, *22*, 36.

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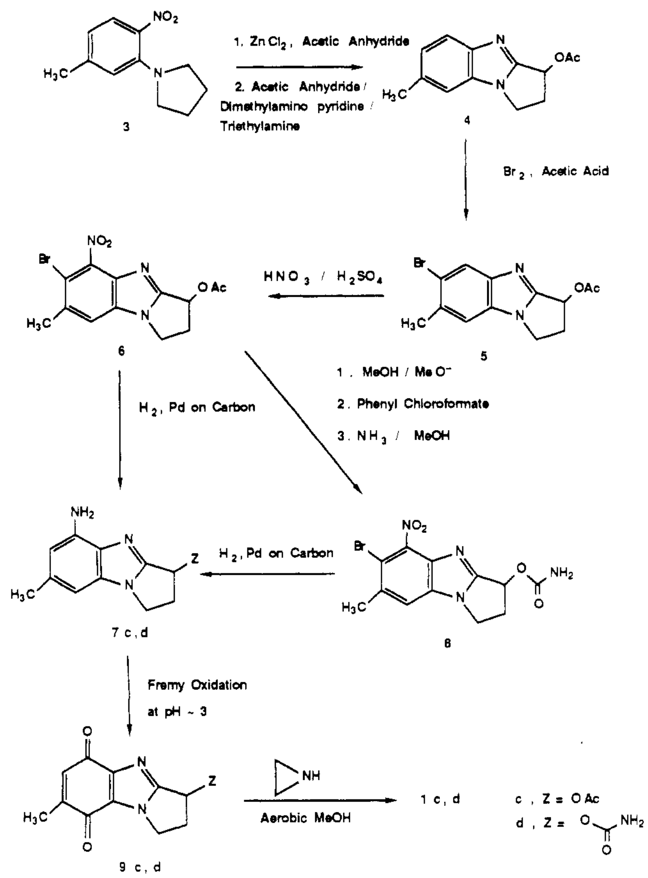
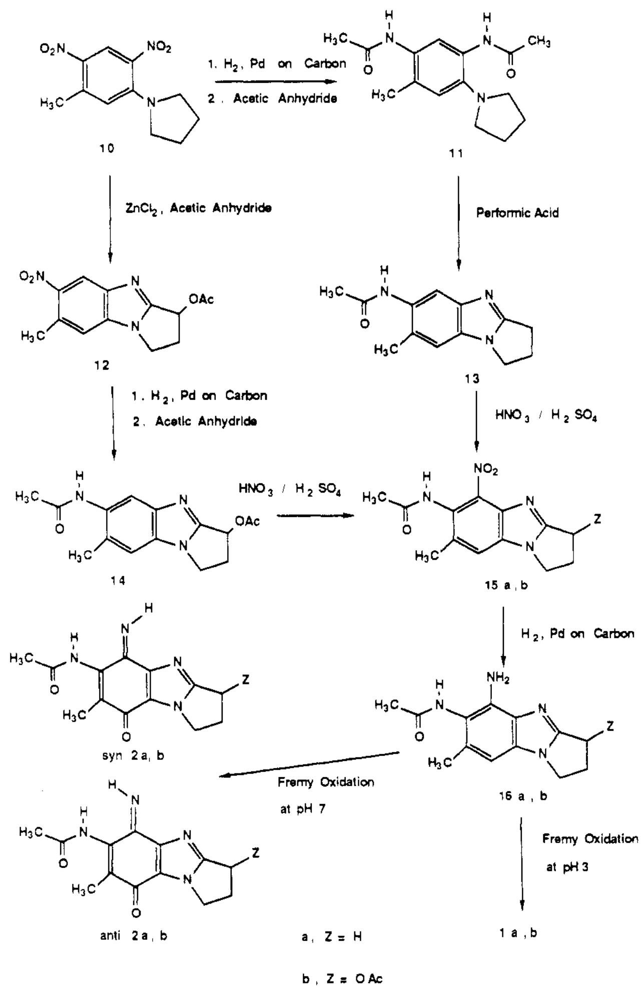
Scheme I



Scheme II



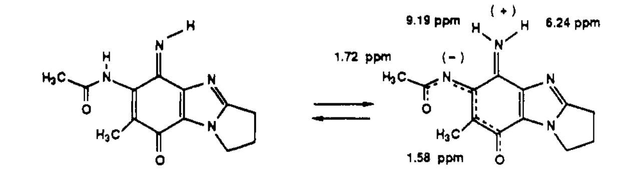
to prepare iminoazamitosenes exhibiting hydrolytic stability and carry out detailed electrochemical and oxygen-reactivity studies.

Presented herein is the synthesis of the azamitosenes 1 and the iminoazomitosenes 2 found in Chart I. The azamitosenes **1c,d** possess alkylating centers at the 3- and 6-positions to permit DNA cross-linking activity. Indeed, preliminary results indicate these compounds are very potent antitumor agents. The iminoazamitosenes 2 are hydrolytically stable below pH 6, and the planned physical studies could be carried out. It is concluded from these studies that the conversion of quinone to iminoquinone is accompanied by an increase in reduction potential as well as a decrease in the oxygen reactivity of the reduced (aminophenol) form.

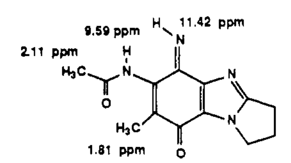
Results and Discussion

Azamitosene and Iminoazamitosene Synthesis. Preparation of the azamitosene ring system (2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole) was carried out either by Lewis acid catalyzed cyclization of an *o*-nitropyrrolidinobenzene derivative (e.g., **3** → **4** and **10** → **12**)[9] or by oxidative cyclization of a diacetamido pyrrolidinobenzene derivative (e.g., **11** → **13**).[10] The former reaction was employed for the preparation of azamitosenes with a leaving group at the 3-position and the latter reaction was employed for the preparation of 3-unsubstituted derivatives.

Scheme III



syn 2a



anti 2a

Quinone and iminoquinone elaboration was carried out by Fremy oxidation[11] of aromatic amine derivatives in pH ~ 3 and in pH 7.0 aqueous buffers, respectively.

Shown in Scheme I is the synthesis of the antitumor agents **1c,d**. The pyrrolo[1,2-*a*]benzimidazole derivative 4 was brominated at the 6-position so as to direct nitration to the 5-position in the next step (**5** → **6**). Catalytic reduction of **6** resulted in both amine reduction and hydrogenolysis of the bromo substituent to afford **7c**. The acetate leaving group of **6** was converted to carbamate (**6**

(9) This reaction is an example of the "*tert*-amino effect": (a) Meth-Cohn, O.; Suschitzky, H. *Adv. Heterocycl. Chem.* **1972**, *14*, 211. (b) Fielden, R.; Meth-Cohn, O; Suschitzky, H. *J. Chem. Soc. Perkin Trans. 1* **1973**, 696, 702, and 705. (c) Grantham, R. K.; Meth-Cohn, O. *J. Chem. Soc. C* **1969**, 70. For more recent work, see: (d) Nijhuis, W. H. N.; Verboom, W.; El-Fadl, A. A.; Harkema, S.; Reinhoudt, D. N. *J. Org. Chem.* **1989**, *54*, 199 and references therein.

(10) (a) Nair, M. D.; Adams, R. *J. Am. Chem. Soc.* **1961**, *83*, 3518. (b) Meth-Cohn, O.; Suschitzky, H. *J. Chem. Soc.* **1963**, 4666.

(11) Zimmer, H.; Lankin, D. C.; Horgan, S. W. *Chem. Rev.* **1971**, *71*, 229.

→ 8) followed by catalytic reduction to afford **7d**. Finally, Fremy oxidation of **7c,d** to **9c,d** and then reductive addition[12] of ethyleneimine in the presence of air afforded **1c,d**.

Shown in Scheme II is the synthesis of the stabilized iminoquinones *syn*-**2a,b** and *anti*-**2a,b**.[13] Fremy oxidation of **16a,b** in pH 7.0 phosphate buffer afforded a syn/anti mixture of iminoquinone isomers,[14] which can be separated by fractional crystallization (see the Experimental Section).

Structural assignments of the isomers were possible using ^{1}H NMR chemical shifts obtained in dimethyl sulfoxide-d_6. The assignments for *syn/anti*-**2a** are discussed in the following paragraph in conjunction with Scheme III. The syn/anti isomers also possess different IR and UV–visible spectra. The latter permitted kinetic studies of the syn/anti isomerization process in aqueous buffer (vide infra). Intramolecular proton transfer only in the syn isomer is likely responsible for all of the observed spectral differences.

The ^{1}H NMR chemical shifts (dimethyl sulfoxide-d_6) of the acetamido methyl and 7-methyl groups of *syn*-**2a** are shifted upfield relative to those of *anti*-**2a**. This observation is consistent with the formation of a delocalized negative charge at the centers bearing the methyl groups in the syn isomer upon intramolecular proton transfer. In contrast, the imino nitrogen lone pair of *anti*-**2a** is anti to the amide proton and a zwitterion cannot form. Nuclear Overhauser effects (NOE) are also consistent with the assigned structures in Scheme III. In the zwitterionic form, the iminium proton at δ 9.19 shows NOE interactions with both the acetamido and 7-methyls while the δ 6.24 iminium proton does not. On the other hand, both nitrogen-substituted protons of *anti*-**2a** show NOE interaction with these methyls. The NOE interactions for the δ 9.59 proton with the methyl groups are much greater than those observed for the δ 11.42 proton, which led to the assignments shown in Scheme III. These assignments are consistent with literature values of imino protons chemical shifts (δ 11.2)[14] and with the acetamido nitrogen proton chemical shifts (δ 7.5–9.3) reported herein (see the Experimental Section).

The IR spectra (KBr pellet) of *syn*- and *anti*-**2a** also supports intramolecular proton transfer in the former compound. The quinone carbonyl stretching frequency of *anti*-**2a** (1683 cm^{-1})[15] is greater than that of *syn*-**2a** (1652 cm^{-1}) due to the decrease in carbonyl bond order in the zwitterion.

Iminoquinone Fate in Aqueous Buffers. The fate of *syn*- and *anti*-**2a**, 6.8×10^{-5} M in aerobic aqueous buffer (μ = 1.0, KCl), was studied at 30 °C over the pH range of 0–9. Outlined in Scheme IV are the pertinent equilibria and hydrolytic reactions of **2a** in aqueous buffer. Above pH 7, the predominate reaction is equilibrium formation of a syn/anti mixture of **2a** by general acid/base-catalyzed processes. Much below pH 7, the predominate reaction is acid-catalyzed hydrolysis of **2a** to the corresponding quinone **1a**. Described below are the studies which led to the mechanism outlined in Scheme IV.

Both hydrolysis and the equilibrium isomerization of pure *syn*-**2a** or pure *anti*-**2a** are associated with an absorbance change at 320 nm. Plots of absorbance vs time

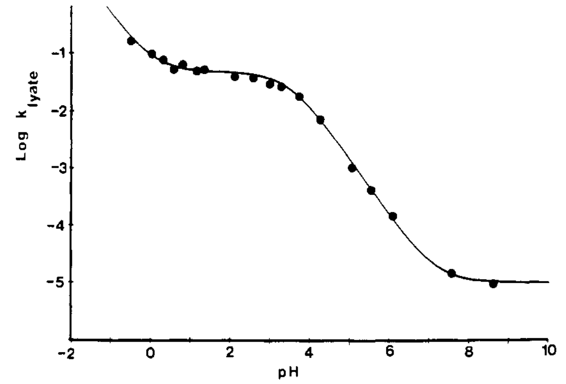
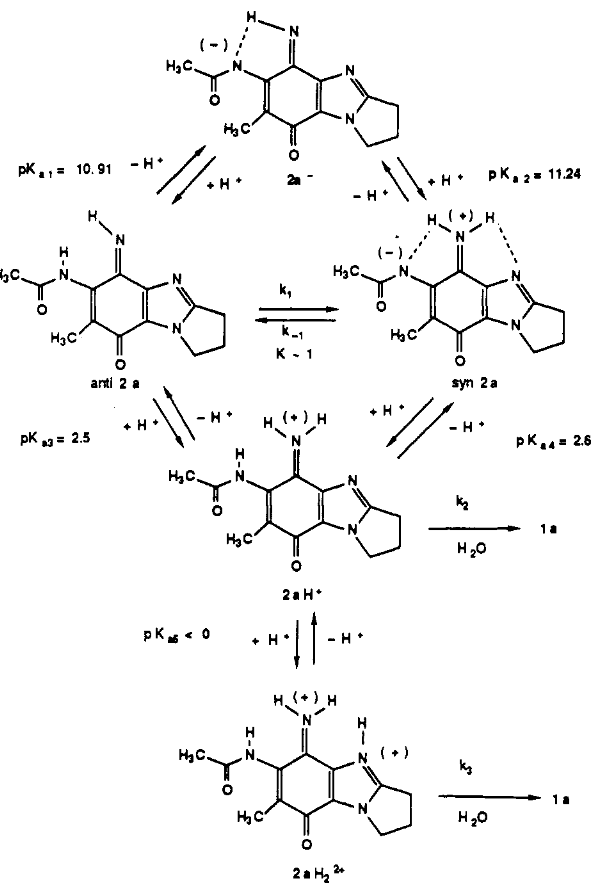


Figure 1. Plot of k_{lyate} vs pH for the first-order reactions (hydrolysis and isomerization) of *syn*-**2a** in aerobic buffer (μ = 1.0, KCl) at 30.0 ± 0.2 °C.

Scheme IV



obeyed a first-order rate law over the entire pH range studied. The k_{obsd} values were dependent on the concentration of the buffers employed to hold pH over the range of pH = 3–9. Lyate-dependent values of k_{obsd} (k_{lyate}) were obtained by measuring k_{obsd} values over a 10-fold range of buffer concentration at constant pH and then extrapolating to the k_{lyate} value at zero buffer concentration. Found in Figure 1 is a plot of the log (k_{lyate}) values vs pH.

Preparative hydrolysis of *syn*-**2a** at pH 4 resulted in the isolation of **1a** in 90% yield. The UV–visible spectra of completed reactions from pH 0 to pH 6 indicated quantitative formation of **1a**. Below pH 7, the preparative reaction of pure *syn*-**2a** afforded a mixture of isomers; [*syn*-**2a**]/[*anti*-**2a**] = 0.7 by ^{1}H NMR.

The pH profile of Figure 1 indicates quinone formation occurs from the monoprotonated and diprotonated forms of **2a**. Thus the plateau (slope −1 to 0) in the acid region

(12) March, L. C.; Joullié, M. M. *J. Het. Chem.* **1970**, *7*, 249.
(13) The syn and anti designations are based on the position of the imine proton relative to the higher ranking substituent (the fused imidazole ring) on the imine carbon.
(14) Helissey, P.; Parrot-Lopez, H.; Renault, J.; Cros, S. *Chem. Pharm. Bull.* **1987**, *35*, 3547.
(15) Bellamy, L. J. *The Infra-red Spectra of Complex Molecules*; John Wiley: New York, 1962; pp 150–151.

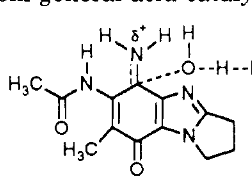
corresponds to the acid dissociation $2aH^+ \rightleftharpoons 2a + H^+$ (pK_a $\sim$ 2.5) and rate-determining hydrolysis of the protonated species. At very high acidity, the rates of hydrolysis again increase with a slope of −1 on the profile, which is attributed to the acid dissociation $2aH_2^{2+} \rightleftharpoons 2aH^+ + H^+$ (pK_a < 0) and rate-determining hydrolysis of the diprotonated species. The small pK_a value of the diprotonated species and the large rates of hydrolysis did not permit the second plateau to be reached, however. Electrochemical studies (vide infra) provided evidence of a diprotonated imine species in strong acid. The pH profile of Figure 1 also indicates the syn/anti equilibration process is either water-catalyzed or spontaneous above pH 7 (i.e., the slope of the profile is zero).

The rate law for the reaction of pure *syn*-**2a**, based on the mechanism shown in Scheme IV, is provided in eq 1 where k_1, k_{-1}, k_2, k_3, K_{a4} and K_{a5} are constants found in Scheme IV and a_H is the proton activity determined with a pH electrode. The first term of eq 1 pertains to the

$$\frac{a_H k_3}{K_{a5}} + \frac{a_H k_2}{a_H + K_{a4}} + (k_1 + k_{-1}) = k_{lyate} \qquad (1)$$

hydrolysis of $2aH_2^{2+}$ under the conditions a_H < pK_{a5}, the second term pertains to hydrolysis of $2aH^+$, and the third term pertains to the syn/anti equilibration. The solid line shown in Figure 1 was computer generated with eq 1 using $k_3/K_{a5} = 4.5 \times 10^{-2}$ M^{-1} s^{-1}, $k_2 = 4.8 \times 10^{-2}$ s^{-1}, pK_{a4} = 3.4, and $k_1 + k_{-1} = 1.03 \times 10^{-5}$ s^{-1}. Consistent with the postulated mechanism, the kinetically obtained value of pK_{a4} approximates the value obtained by spectrophotometric titration (2.6 ± 0.3).

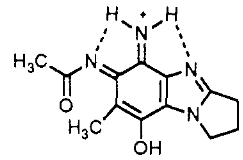
The hydrolysis of **2a** is also subject to general acid catalysis over the range pH 3.5 to 6; k_{ga} for acetic acid was found to be 3×10^{-3} M^{-1} s^{-1}. Since **2a** is largely protonated at the low end of this pH range, general acid catalysis must not pertain to rate-determining protonation of the imine nitrogen. An alternative mechanism is general-base-catalyzed addition of water to the protonated imine as shown below, structure **17**. This mechanism is a specific acid/general base catalyzed process, which is kinetically indistinguishable from general acid catalysis.[16]



17

The mechanism of **2a** hydrolysis discussed in the preceding paragraphs is typical of electron-deficient imines[17] and other iminoquinones.[18] Thus, water addition to the protonated imine is rate determining and general-base catalyzed. The high reduction potential of **2a** indicates it is an electron-deficient system (vide infra, Electrochemistry Section).

Factors which may influence the hydrolytic stability of **2a** below pH 6 include its electron-deficient character as well as stabilization of the protonated imine by internal hydrogen bonding. The former results in a low pK_a for acid dissociation of the protonated imine ($\sim$2.5) and, consequently, the presence of very little protonated species at neutrality. Internal hydrogen-bonding interactions in the structure shown below would diminish the positive charge on the imine nitrogen and slow water addition. The pK_a of $2aH^+$ ($\sim$2.5) and the slow rate of water addition to this species (4.8×10^{-2} s^{-1}) indicate hydrolysis would only occur at 1.0×10^{-6} s^{-1} at pH = 7.0 ($t_{1/2}$ $\sim$ 5 days).

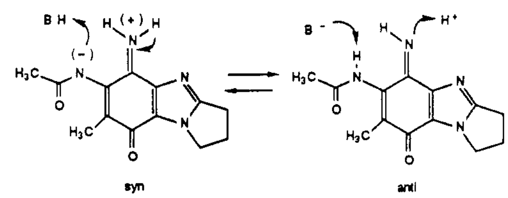


18

Evidence of internal hydrogen bonding involving the enolized amide nitrogen was obtained from thermodynamic studies of the syn/anti isomerization (vide infra, this section). Furthermore, previous work in this laboratory suggested the formation of enols of this type in aqueous solution.[19]

Kinetic and thermodynamic aspects of the syn/anti isomerization process are discussed in the following paragraphs.

Both general-acid and general-base catalysis are observed over the pH range where syn/anti imine isomerization occurs: k(acetate) = 4.5×10^{-3} M^{-1} s^{-1}, k(monobasic phosphate) = 4.21×10^{-4} M^{-1} s^{-1}, and k(dibasic phosphate) = 1.71×10^{-4} M^{-1} s^{-1}. The presence of general catalysis suggests the isomerization mechanism involves prototropic shifts as shown in eq 2. Others have also postulated the prototropic syn/anti isomerization of unsubstituted imines.[20,21]



(2)

The Principle of Microreversibility requires general-base catalysis in one direction and general-acid catalysis in the opposite direction during the syn/anti equilibration process shown in eq 2. The rate law for the equilibration process is thus

$$k_{syn/anti} = k_1 + k_{-1} + k_{gb}[B] + k_{ga}[BH] \qquad (3)$$

where k_1 and k_{-1} (Scheme IV) are water-catalyzed (lyate) rates and k_{gb} and k_{ga} are general base and general acid catalyzed rates, respectively. As required by eq 3, equilibration actually involves both general acid and base catalysis.

The K value ($\sim$1) for syn/anti isomerization of **2a** in aqueous buffer was assessed from three thermodynamic cycles and from a product study. The results of the product study (loc cit, this section) indicate K = 0.7. The two thermodynamic cycles shown in Scheme IV indicate K = 1–2. These thermodynamic cycles were constructed by considering that protonation of *syn*/*anti*-**2a** provides the same cationic species $2aH^+$ and that acid dissociation from *syn*/*anti*-**2a** probably provides the same anionic species $2a^-$. The third thermodynamic cycle was obtained

(16) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; McGraw-Hill: New York, 1969; p 184.

(17) Riemann, J. E.; Jencks, W. P. *J. Am. Chem. Soc.* **1966**, *88*, 3973. Also see ref 16, pp 490–496.

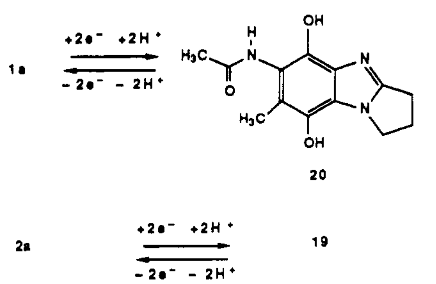
(18) Brown, E. R. In *The Chemistry of the Quinonoid Compounds*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1988; Vol. 2, Part 2, Chapter 21.

(19) Skibo, E. B. *J. Org. Chem.* **1985**, *50*, 4861.

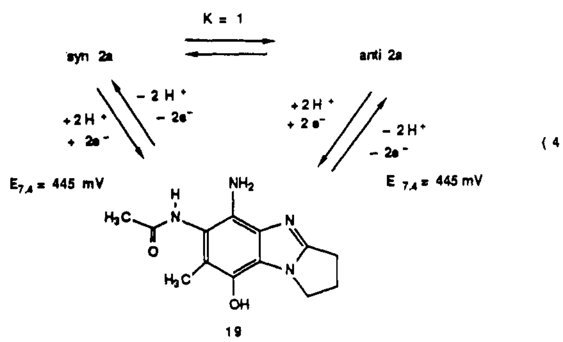
(20) Lambert, J. B.; Oliver, W. L.; Roberts, J. D. *J. Am. Chem. Soc.* **1965**, *87*, 5085.

(21) For a review of syn/anti imine isomerization see: McCarty, C. G. In *The Chemistry of the Carbon–Nitrogen Double Bond*; Patai, S., Ed.; Interscience: New York, 1970; Chapter 9.

Chart II



by cyclic voltammetry; identical E values for both isomers indicate equilibration is a thermoneutral process, eq 4.



(4)

The thermoneutral syn/anti isomerization in aqueous buffer requires that the imino proton of *anti*-**2a** hydrogen bond to the nitrogen of the enolized acetamido group. In the absence of this hydrogen bond, *syn*-**2a** would likely be more stable than *anti*-**2a** in aqueous solution due to the presence of one or more internal hydrogen bonds in the former isomer (see *syn*-**2a** in Scheme IV). Our ^{1}H NMR and NOE studies of *anti*-**2a** in dimethyl sulfoxide-d_6 indicate enolization of the 6-acetamido group does not occur and thus *syn*-**2a** should be more stable than *anti*-**2a** in this solvent since no internal hydrogen bonds can stabilize the anti isomer. Indeed, the K for syn/anti equilibration in this solvent is 15.

The conclusion of the iminoquinone hydrolytic studies is that internal hydrogen bonding is responsible for both the iminoquinone stability and the K value for syn/anti isomerization. Significantly, internal hydrogen bonding is also responsible for the hydrolytic stability of iminodaunomycin[8] as well the as electrochemical properties of other iminoquinones.[22]

Electrochemistry. In this section, comparisons are made of the quinone and iminoquinone two-electron couples shown in Chart II. The oxygen reactivity of the respective reduced forms of these couples, **20** and **19**, is also compared. It is concluded that the change from quinone to iminoquinone is accompanied by increases in reduction potential as well as decreases in oxygen-mediated reoxidation rates of the reduced species.

Quinone **1a** and iminoquinone **2a** two-electron reduction potentials were determined in anaerobic aqueous buffers (μ = 1, $NaClO_4$) at 25 °C over the pH range -1 to 10 employing conventional cyclic voltammetry. The working electrode was a graphite mull, the auxilliary electrode was platinum, and the reference couple was Ag/AgCl. The voltammograms are quasireversible in character and also show a high degree of symmetry ($\alpha \sim 0.5$).[23]

Voltammograms of the couple **1a/20** were obtained by scanning solutions of **1a** in the cathodic and then anodic directions (300 mV s^{-1}). The quinone **1a** is hydrolytically stable throughout the entire pH range studied, and aqueous solutions could be prepared and degassed without appreciable decomposition. The iminoquinone **2a** is hydrolytically stable at pH values ≥7, and voltammograms were also obtained by cathodic–anodic scanning of solutions of the oxidized species. Much below pH 6, **2a** is rapidly hydrolyzed to the quinone, and it was necessary to do anodic–cathodic scans on solutions of the acid-stable reduced species **19**. Fast scans (>1000 mV s^{-1}) of acid solutions of **19** provided quasireversible voltammograms of **2a/19** even though **2a** rapidly hydrolyses in these solutions.

Found in Figure 2 are E_m vs pH data for both couples along with solid lines computer generated from the Nernst equation. Fitting the E_m vs pH data in Figure 2 to the Nernst equation requires two more acid dissociations in the reduced species than the oxidized species. Tabulated in Table I are the pK_a values of **1a/20** and of **2a/19** used in the Nernst fits. The pK_a values with error limits were determined spectrophotometrically, and the other pK_a values, which fall outside the pH range studied, are approximate values.

The Nernst fits in Figure 2 show that the iminoquinone

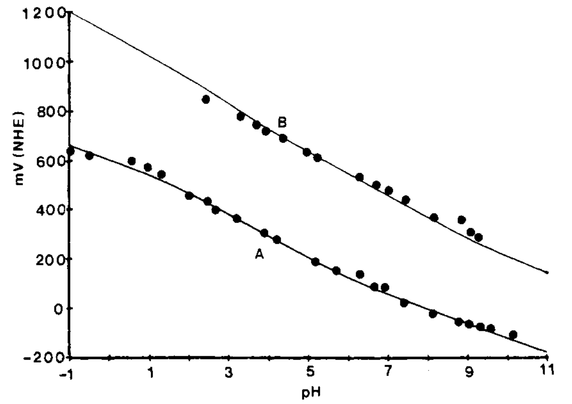


Figure 2. E_m vs pH data for the two-electron couples **2a/19** (plot B) and **1a/20** (plot A) measured at 25–26 °C in anaerobic buffer (μ = 1.0, $NaClO_4$). The solid curves were generated by employing the Nernst equation.

Table I. pK_a Values of 1a/20 and 2a/19 Determined at 30 °C in μ = 1.0 (KCl) Buffer

entry	acid dissociation	acid species	pK_a
1	$1aH^+ \rightleftharpoons 1a + H^+$	N(4)-protonated quinone	1.54 ± 0.06
2	$1a \rightleftharpoons 1a^- + H^+$	amide proton of quinone	11.32 ± 0.13
3	$20H^+ \rightleftharpoons 20 + H^+$	N(4)-protonated hydroquinone	6.02 ± 0.20
4	$20 \rightleftharpoons 20^- + H^+$	5-OH of hydroquinone	11.11 ± 0.19
5	$20^- \rightleftharpoons 20^{2-} + H^+$	8-OH of hydroquinone	>14
6	$20^{2-} \rightleftharpoons 20^{3-} + H^+$	amide proton of hydroquinone	>14
7	$2aH_2^{2+} \rightleftharpoons 2aH^+ + H^+$	N(4)-protonated iminoquinone	<0
8	$2aH^+ \rightleftharpoons 2a + H^+$	protonated imino group	2.6 ± 0.3
9	$2a \rightleftharpoons 2a^- + H^+$	amide proton of iminoquinone	11.24 ± 0.01
10	$19H_2^+ \rightleftharpoons 19H^+ + H^+$	N(4)-protonated aminophenol	3.67 ± 0.16
11	$19H^+ \rightleftharpoons 19 + H^+$	protonated 5-amino group	9.44 ± 0.04
12	$19 \rightleftharpoons 19^- + H^+$	dissociation of hydroxyl, amide and amine protons	>15
13	$19^- \rightleftharpoons 19^{2-} + H^+$		>15
14	$19^{2-} \rightleftharpoons 19^{3-} + H^+$		>15

(22) Amatore, C.; Anne, A.; Florent, J. C.; Moiroux, J. *J. Electroanal. Chem.* **1986**, *207*, 151.
(23) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley: New York, 1980; pp 227-231.

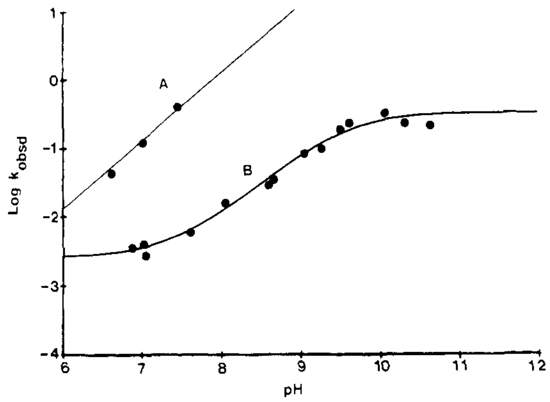


Figure 3. Plots of k_{obsd} vs pH for the aerobic oxidation of **19** (plot B) and **20** (plot A) in aqueous buffer (μ = 1.0, KCl) at 30.0 ± 0.2 °C.

(plot B) possesses much higher potentials than the quinone derivative (plot A). For example, the E_o value (reduction potential at pH = 0) for **2a**/**19** is 1.11 V (NHE) and the E_o value for **1a**/**20** is only 612 mV (NHE). Indeed, the iminoquinone even possesses an E_o value greater than dichlorodicyanoquinone (DDQ) (E_o = 946 mV, NHE).[24] Like DDQ,[25] **2a** rapidly hydrolyses to a lower potential species (**1a**) in strong acid. Inspection of the pK_a data in Table I reveals the reason of the high iminoquinone reduction potentials. Over the entire pH range studied, the iminoquinone couple **2a**/**19** is either diprotonated or monoprotonated whereas the quinone couple **1a**/**20** is either monoprotonated or neutral. Electron-deficient couples, resulting from the presence of electron-withdrawing groups or protonation, possess high reduction potentials.[26]

In the absence of protic equilibria, iminoquinones should possess lower reduction potentials than quinones. This assessment is based on the lower electronegativity of nitrogen compared to oxygen. In fact, iminoanthraquinones possess lower reduction potentials for single-electron transfer in aprotic solvent than the quinone analogues.[22]

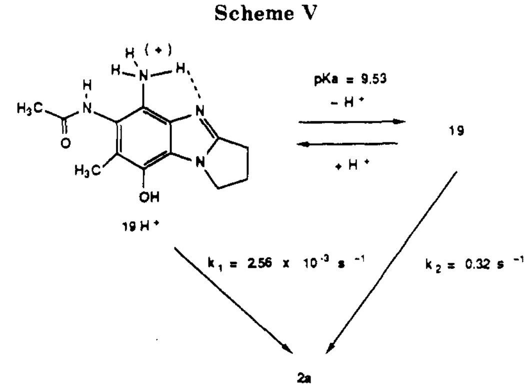
The oxygen reactivity of **19** and **20** was studied in aerobic buffers (μ = 1.0, KCl) at 30 °C. Both of these species are converted to the corresponding oxidized analogues, **2a** and **1a**, by first-order processes. Isolation studies and UV-visible spectra of completed reactions confirmed the formation of these products. As expected from the relative reduction potentials, the oxidation of **19** is a slow enough to be studied up to pH 11 whereas the oxidation of **20** occurs at stopped flow rates much above neutrality.

Shown in Figure 3 are the pH profiles for the oxidation of **19** (plot B) and **20** (plot A). The profile for the oxidation of **19** is consistent with oxidation of both the neutral species (**19**) and the monoprotonated species (**19H⁺**), Scheme V. The rate law for the mechanism in Scheme V is found in eq 5 where k_1 and k_2 are apparent first-order rate constants containing a term for the partial pressure of oxygen, K_a is the acid dissociation constant of **19H⁺**, and a_H is the proton activity determined with a pH electrode.

$$k_{obsd} = k_1 + \frac{k_2K_a}{a_H + K_a} \quad (5)$$

The solid curve of plot B in Figure 3 was computer generated with eq 5 using the values of the constants in

Scheme V



Scheme V. Consistent with the proposed mechanism, the kinetically determined value of pK_a is nearly the same as the value determined spectrophotometrically (9.44, entry 11 of Table I). The greater oxygen reactivity of **19**, compared to **19H⁺**, is consistent with the decreasing E_m values (i.e., decreasing stability of the reduced species) observed in the Nernst Fit (plot B, Figure 2) at pH values > 6.

The oxidation rates of **20** increase with pH (slope of +1, plot A of Figure 3) and become too fast to measure above pH 7.5. In the pH range studied, **20** is largely in the neutral form with only small amounts of the hydroxyl anion **20⁻** present (pK_a = 11.11, Table I). The positive slope of plot A may pertain to equilibrium formation of **20⁻** (**20** ⇌ **20⁻** + H⁺) and rate-determining oxidation of this species. If this is the case, **20⁻** is oxidized to **1a** at 1566 s^{-1} in aerobic buffer.

Comparison of the pH profiles in Figure 3 indicates **19** is oxidized about 100 times slower than **20** at physiological pH (7.4). Thus, iminoquinone-based reductive alkylating agents should generate significantly less toxic oxygen species than quinone-based agents.

Conclusions

The synthesis of pyrrolo[1,2-*a*]benzimidazole (azamitosene) reductive alkylating agents is described. Analogues **1c,d** are potent antitumor agents: IC_{50} values as low as 0.6 nM in cloned human ovarian and colon cancer cell lines.[27] Antitumor activity could pertain to DNA monoalkylation as well as DNA cross-linking. Details of structure–activity studies will be reported in due course[28] as will the results of DNA alkylation studies.

The synthesis of iminoquinone derivatives (iminoazamitosenes) **2a,b** is also described. Internal hydrogen bonding involving the 6-acetamido and the 4-nitrogen of the pyrrolo[1,2-*a*]benzimidazole ring serves to stabilize the imine group so that hydrolysis below pH 6 is extremely slow (see Figure 1). In fact, the attempted preparation of the 6-unsubstituted iminoquinone by Fremy oxidation of **7** (Scheme I) at pH 7 afforded only the quinone **9**. Internal hydrogen bonding also influences the thermodynamics of buffer-catalyzed imine syn/anti isomerization. We conclude from our synthetic and physical studies that a variety of hydrolytically stable iminoquinone reductive alkylating agents can be prepared. These iminoquinones will hydrolyze only under acidic conditions, and hydrolytic stability is expected in a variety of cellular environments.[29]

Our electrochemical studies indicate iminoquinone reductive alkylating agents will possess low oxygen toxicity.

(24) Skibo, E. B.; Gilchrist, J. H. *J. Org. Chem.* **1988**, *53*, 4209.

(25) Becker, H.-D.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1987**, *40*, 625.

(26) Becker, H.-D. In *The Chemistry of the Quinonoid Compounds*; Patai, S., Ed.; Wiley: New York, 1974; Part I, Chapter 7.

(27) Studies carried out in the laboratory Professor David S. Alberts, Arizona Cancer Center, Tucson, Arizona.

(28) The antitumor studies will be reported from this and Professor Alberts' laboratory.

(29) Healthy cells possess a pH value of 7.3 while cancer cells possess pH values as low as 6: Albert, A. *Selective Toxicity*, 6th ed.; Chapman and Hall: London, 1979; p 142.

Iminoquinone **2a** possesses much higher reduction potentials than its quinone analogue **1a**. The reduced form of **2a** (**19**) thus reoxidizes 2 orders of magnitude slower than the reduced form of **1a** (**20**) in aerobic buffer at physiological pH. In contrast, iminodaunomycin has low oxygen toxicity as a result of reductive deamination to afford a high reduction potential species unable to generate oxygen radicals efficiently.[30]

Experimental Section

All analytically pure compounds were dried under high vacuum *at room temperature* or *in a drying pistol heated with refluxing* methanol. Compounds susceptible to decomposition (**1c**,**d**, **2**, **19**, **20**) were not heated above room temperature. Some of the compounds still contained water of crystallization that was determined from the elemental analyses found. Experimental nitrogen percentages for **1c**,**d** and *syn*-**2a** deviated from theoretical percentages by >0.5%. Repeat nitrogen analyses often showed a wide variation in percentage values; we believe this is due to incomplete combustion. ^{1}H NMR and ^{13}C NMR data and mass spectra (both the parent ion and fragmentation pattern) supported the assigned structures, and TLC indicates these compounds are pure. No elemental analyses were obtained for *anti*-**2a**,**b**, **7c**,**d**, **14**, **19**, **20**; spectral data support the assigned structures, and these compounds can be converted to well-characterized compounds.

Uncorrected melting and decomposition points were determined with a Mel-Temp apparatus. All TLC was run with Merck silica gel 60 (F_{254}) plates, employing a variety of solvents. IR spectra were taken as KBr pellets or thin films; the strongest IR absorbances are reported. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AM-400 spectrometer, and chemical shifts are reported relative to TMS.

pK_a constants were determined by spectrophotometric titration in μ = 1.0 (KCl) aerobic aqueous solvent at 30 ± 0.2 °C with a Cary 15 instrument outfitted with a titration cell. Acid dissociations from hydroquinones in strong base were measured under an argon atmosphere with Thunberg cuvettes. Details of the methodology employed are found in a previous publication.[31]

Kinetic Studies of Hydrolysis. The hydrolytic studies of **2a** and the reoxidation studies of **19** and **20** were carried out in aerobic aqueous buffer at 30.0 ± 0.2 °C. A dimethyl sulfoxide stock of the compound to be studied was prepared fresh, and 50 μL of this stock was added to 2.95 mL of buffer. In the cases of **19** and **20**, the dimethyl sulfoxide stock was kept under a blanket of argon. The absorbance vs time data were obtained on a Perkin-Elmer 559 or a Lambda-3 UV-vis spectrophotometer and fit to a first-order rate law.

Electrochemistry. The determination of E_m values was carried out with a BAS 27 voltammograph. Measurements were carried out in μ = 1.0 ($NaClO_4$) aqueous buffer at 25–26 °C under an atmosphere of argon with a BAS Ag/AgCl gel electrode as reference. The electrode was calibrated against the E_o value of the benzoquinone/hydroquinone couple (699 mV, NHE).[32] The midpoint potential E_m was determined from the average of the anodic ($E_{p,a}$) and cathodic ($E_{p,c}$) potentials.

Nernst Fit. For each of the redox couples, **1a**/**20** and **2a**/**19**, >20 E_m determinations were made over the pH range studied. For each E_m value of a couple, an E_o value was calculated from the Nernst equation[33] substituted with the acid dissociation constants in Table I and the proton activity determined with a pH meter. The average of all E_o determinations was then substituted into the Nernst equation, with which the solid curve for the couple was generated.

Product Isolation of *syn*-2a Hydrolysis at pH 4. To a solution 11 mg (0.042 mmol) of *syn*-**2a** in 1 mL of dimethyl sulfoxide was added 4 mL of pH 4 acetate buffer. The reaction mixture was stirred for 35 min at room temperature and then extracted three times with 10-mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated to a solid, which was recrystallized from chloroform/hexane, yield of **1a** was 10 mg (90%). Identity as **1a** was based on ^{1}H NMR, mass spectral, and TLC data.

Product Isolation of *syn*-2a Reaction at pH 8. To a solution of 15 mg (0.058 mmol) of *syn*-**2a** in 1 mL of dimethyl sulfoxide was added 4 mL of pH 8 phosphate buffer. The reaction mixture was stirred at room temperature for 48 h and then diluted with 10 mL of water and extracted three times with 20-mL portions of chloroform. Concentration of the dried extracts (sodium sulfate) afforded 12 mg (80%) of a mixture of isomers: [syn]/[anti] = 0.7 by ^{1}H NMR.

Product Isolation of Aerobic Oxidation of 19. To a solution of 15 mg (0.057 mmol) of **19** in 0.5 mL of dimethyl sulfoxide was added 6.0 mL of pH 9 borate buffer. After the reaction was stirred at room temperature for 15 min, 10 mL of water was added, and the diluted solution was extracted with 3 × 25-mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated, and the residue recrystallized from chloroform/hexane. Yield of a syn/anti mixture of **2a** was 5 mg (33%). Identity was based on ^{1}H NMR and mass spectral data.

Synthesis and physical properties of new compounds are provided below:

3-(*N*-Pyrrolidino)-4-nitrotoluene (3). A mixture of 8.64 g (40 mmol) of 3-bromo-4-nitrotoluene[34] and 8.5 g (120 mmol) of pyrrolidine was heated at reflux for 3 h. The cooled reaction mixture was poured over 200 g of cracked ice, and the resulting mixture was extracted two times with 200-mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated to an oily residue, which was placed on a silica gel flash column. The product was eluted with hexane/chloroform (50:50). Evaporation of the eluant afforded an orange oil, which slowly solidified upon chilling in a refrigerator: yield 7.8 g (94%); mp 42 °C; TLC ($CHCl_3$) R_f = 0.46; IR (film on NaCl) 1612, 1569, 1500, 1465, 1447, 1430, 1360, 1356, 1274, 600 cm^{-1}; NMR ($CDCl_3$) δ 6.52 and 7.66 (2 H, ABX, J_{ortho} = 8.24 Hz, J_{meta} = 1.3 Hz, J_{para} ~ 0 Hz, C(5) and C(6) aromatic protons, respectively), 6.69 (1 H, br s, C(2) aromatic proton), 3.20 (4 H, m, pyrrolidine methylenes adjacent to N), 2.34 (3 H, s, methyl), 1.97 (4 H, m, other pyrrolidine methylenes); mass spectrum (EI mode), *m/z* 206 (P^+). Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.05; H, 6.84; N, 13.58. Found: C, 63.49; H, 6.73; N, 13.32.

7-Methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Acetate (4). A mixture consisting of 2.06 g (10 mmol) of **3**, 1.36 g (10 mmol) of anhydrous $ZnCl_2$, and 10 mL of acetic anhydride was stirred at 100–110 °C for 5 h (or until **3** was no longer seen by TLC). The reaction mixture was poured into 10 mL of water, and the black oil which formed was separated and evaporated to a small volume. The residue was combined with 20 mL of concentrated HCl and warmed to 80 °C for 5 min. Hydrogen sulfide gas was then passed into the HCl solution for 5 min followed by addition of NaOH until the pH = 6.5–7.0. Extraction of the above mixture with 3 × 50-mL portions of chloroform, drying the extracts (sodium sulfate), and chromatography on silica gel (column prepared with chloroform and the product eluted with chloroform/methanol [95:5]) afforded the 3-hydroxy derivative of **4** as a white powder: 1.00 g (52%) yield; mp 212 °C; TLC (chloroform/methanol [90:10]) R_f = 0.52; IR (KBr pellet) 3135, 2861, 1524, 1445, 1350, 1322, 1298, 1290, 1092, 816 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.46 and 6.98 (2 H, ABX system, J_{ortho} = 8.2 Hz, J_{meta} = 1.20 Hz, J_{para} ~ 0 Hz, C(5) and C(6) protons, respectively), 7.26 (1 H, br s, C(8) proton), 5.78 (1 H, d, J = 6.0 Hz, 3-hydroxyl proton), 5.05 (1 H, m, C(3) proton), 4.15 and 3.99 (2 H, 2 m, C(1) diastereomeric methylene), 2.88 and 2.36 (2 H, 2 m, C(2) diastereomeric methylene), 2.40 (3 H, s, 7-methyl); mass spectrum (EI mode), *m/z* 188 (P^+), 171 (P^+ − OH). Anal. Calcd for $C_{11}H_{12}N_2O$: C, 70.21; H, 6.38; N, 14.89. Found: C, 69.84; H, 6.33; N, 14.82.

Acetylation of the alcohol obtained above was carried out by stirring a mixture consisting of 376 mg (2 mmol) of the alcohol, 224 mg (2.07 mmol) of acetic anhydride, 122 mg (1 mmol) of

(30) Bird, D. M.; Boldt, M.; Koch, T. H. *J. Am. Chem. Soc.* **1987**, *109*, 4046.
(31) Skibo, E. B.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 3304.
(32) Conant, J. B.; Fieser, L. F. *J. Am. Chem. Soc.* **1923**, *45*, 2194.
(33) (a) Clark, W. M. *Oxidation Reduction Potentials of Organic Systems*; Williams and Wilkins: Baltimore, 1960; p 118. (b) Eberlein, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 6685.

(34) (a) Elson, L. A.; Gibson, C. S.; Johnson, J. D. A. *J. Chem. Soc.* **1929**, 2735. (b) Blackburn, W.; Danzig, M.; Hubinger, H.; Soisson, D.; Schultz, H. P. *J. Org. Chem.* **1961**, *26*, 2805.

(dimethylamino)pyridine, 220 mg (2.2 mmol) of triethylamine, and 20 mL of methylene chloride for 30 min at room temperature. The reaction mixture was then washed with water (3 × 25 mL) and dried over sodium sulfate. Evaporation of mixture to an oil, and trituration with chloroform/hexane afforded **4** as a white solid: 391 mg (85%) yield; mp 154 °C; TLC (chloroform/methanol [90:10]) R_f = 0.76; IR (KBr pellets) 1747, 1537, 1427, 1372, 1291, 1269, 1251, 1224, 1053, 808 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.50 and 7.04 (2 H, ABX system, J_{ortho} = 8.3 Hz, J_{meta} = 1.3 Hz, J_{para} ~ 0 Hz, C(5) and C(6) aromatic protons, respectively), 7.26 (1 H, br s, C(8) aromatic proton), 6.10 (1 H, dd, J = 7.6 Hz, J = 3.3 Hz, C(3) proton), 4.22 and 4.12 (2 H, 2 m, C(1) diastereomeric methylene), 3.10 and 2.56 (2 H, 2 m, C(2) diastereomeric methylene), 2.43 (3 H, s, 7-methyl), 2.07 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 230 (P$^+$), 187 (P$^+$ − acetyl). Anal. Calcd for $C_{13}H_{14}N_2O_2$: C, 67.88; H, 6.12; N, 12.16. Found: C, 67.26; H, 5.99; N, 11.94.

6-Bromo-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Acetate (5). To a solution of 500 mg (2.17 mmol) of **4** in 10 mL of glacial acetic acid, heated at 100 °C, was added 3 mL of 0.72 M bromine in glacial acetic acid. After the addition, the reaction mixture was heated at 100–110 °C for 4 h. The cooled reaction mixture was diluted with 20 mL of water and then neutralized to pH 6.5 with aqueous sodium bicarbonate. The product crystallized from the solution as white crystals; yield upon drying the collected solid was 510 mg (75%). Recrystallization from chloroform/hexane afforded analytically pure material: mp 191 °C dec; TLC (chloroform/methanol [80:20]) R_f = 0.64; IR (KBr pellet) 1748, 1531, 1455, 1424, 1371, 1288, 1249, 1082, 1051, 851 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.86 and 7.58 (2 H, 2 s, aromatic protons), 6.11 (1 H, dd, J = 7.6 Hz, J = 3.2 Hz, C(3) proton coupled to C(2) methylene) 4.23 and 4.12 (2 H, 2 m, C(1) diastereomeric methylene) 3.12 and 2.52 (2 H, 2 m, C(2) diastereomeric methylene), 2.49 (3 H, s, 7-methyl), 2.07 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 308 and 310 (P$^+$, ^{79}Br and P$^+$, ^{81}Br), 265 and 267 (P$^+$ − acetyl) 249 and 251 (P$^+$ − acetic acid). Anal. Calcd for $C_{13}H_{13}BrN_2O_2 \cdot 0.25H_2O$: C, 49.76; H, 4.25; N, 8.92. Found: C, 50.00; H, 4.20; N, 8.85.

6-Bromo-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Acetate (6). A solution of 500 mg (1.61 mmol) of **5** in 10 mL of a 9:1 mixture of fuming nitric acid and concentrated sulfuric acid was stirred in an ice bath for 10 min. The completed reaction was poured over cracked ice, and the pH of the resulting solution was adjusted to pH 6.5 with aqueous sodium bicarbonate. Extraction of this solution with 3 × 50 mL of chloroform, drying the extracts (sodium sulfate), and then concentration afforded a yellow oil. Dissolution of this oil in a small volume of chloroform and addition of hexane resulted in crystallization of **6**: 411 mg (71%) yield; mp 185 °C dec; TLC (chloroform/methanol [80:20]) R_f = 0.73; IR (KBr pellet) 1747, 1539, 1488, 1442, 1374, 1350, 1305, 1232, 1089, 1044 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.90 (1 H, s, aromatic), 6.15 (1 H, dd, J = 7.7 Hz, J = 3.2 Hz, C(3) proton coupled with C(2) methylene), 4.33 and 4.20 (2 H, 2 m, C(1) diastereomeric methylene), 3.15 and 2.60 (2 H, 2 m, C(2) diastereomeric methylene), 2.55 (3 H, s, 7-methyl), 2.09 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 353 and 355 (P$^+$, ^{79}Br and P$^+$, ^{81}Br), 310 and 312 (P$^+$ − acetyl), 293 and 295 (P$^+$ − acetic acid). Anal. Calcd for $C_{13}H_{12}BrN_3O_4$: C, 44.07; H, 3.41; N, 11.86. Found: C, 44.16; H, 3.27; N, 11.59.

6-Bromo-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Carbamate (8). The conversion of **6** to **8** was carried out by the three-step process described below.

Deacetylation was carried out by suspending 200 mg (0.56 mmol) of **6** in 25 mL of methanol and then adding 31 mg of sodium methoxide. The reaction was stirred for 30 min at room temperature, and the crystallized alcohol derivative was filtered off; 142 mg (81%) yield. Recrystallization was carried out by dissolving the product in 15 mL of methanol–chloroform (1:4) and then adding a small amount of hexane followed by chilling: mp 255 °C dec; TLC (chloroform/methanol [90:10]) R_f = 0.4; IR (KBr pellet) 3200, 1545, 1516, 1438, 1381, 1372, 1345, 1299, 1101 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.83 (1 H, s, aromatic), 6.03 (1 H, d, J = 5.6 Hz, 3-hydroxyl), 5.13 (1 H, m, C(3) proton), 4.27 and 4.09 (2 H, 2 m, C(1)-diastereomeric protons), 2.94 and 2.41 (2 H, 2 m, C(2)-diastereomeric protons), 2.53 (3 H, s, 7-methyl); mass spectrum (EI mode), m/z 311 and 313 (P$^+$, ^{79}Br and P$^+$, ^{81}Br). Anal. Calcd for $C_{11}H_{10}BrN_3O_3$: C, 42.31; H, 3.22; N, 13.46. Found: C, 42.44; H, 3.13; N, 13.34.

The phenyl carbonate derivative of the alcohol was prepared as described below. To a solution of the alcohol (400 mg, 1.27 mmol) in 20 mL of pyridine, chilled to 0 °C, was added 400 μL of phenyl chloroformate. The reaction was stirred at 0 °C for 15 min and then at room temperature for 1 h. The completed reaction was diluted with 150 mL of ethyl acetate, and the resulting mixture was extracted three times with 50 mL of 20% acetic acid and then two times with 50 mL of water. Drying of the extracts (sodium sulfate) and concentration afforded the carbonate as a light yellow solid; yield 450 mg (81%). Recrystallization was carried out from chloroform/hexane: mp 172–175 °C; TLC (chloroform/methanol [90:10]) R_f = 0.64; IR (KBr pellet) 1765, 1538, 1350, 1293, 1249, 1201, 1184, 1084, 946, 777 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.94 (1 H, s, aromatic), 7.46 and 7.31 (5 H, 2 m, phenyl), 6.22 (1 H, dd, J = 7.5 Hz, J = 3.6 Hz, C(3) proton), 4.39 and 4.26 (2 H, 2 m, C(1) diastereomeric methylene), 3.24 and 2.85 (2 H, 2 m, C(2) diastereomeric methylene), 2.56 (3 H, s, 7-methyl); mass spectrum (EI mode), m/z 431 and 433 (P$^+$, ^{79}Br and P$^+$, ^{81}Br), 294 and 296 (P$^+$ − $PhOCO_2$). Anal. Calcd for $C_{18}H_{14}BrN_3O_5 \cdot 0.25H_2O$: C, 49.49; H, 3.28; N, 9.61. Found: C, 49.62; H, 3.17; N, 9.51.

The preparation of the carbamate **8** was carried out by treatment of the carbonate derivative with ammonia. To 30 mL of anhydrous ammonia at −76 °C was added a solution of the carbonate, 211 mg (0.48 mmol), in 30 mL of dry dichloromethane. The solution was stirred at −76 °C for 30 min, and the reaction was allowed to come to room temperature over a 3-h period. The solvent was evaporated, and the solid residue was recrystallized from chloroform/hexane to afford yellow crystals of **8**: 150 mg (86%) yield; mp 236 °C dec; TLC (chloroform/methanol [90:10]) R_f = 0.4; IR (KBr pellet) 3372, 1715, 1533, 1416, 1400, 1378, 1370, 1335, 1300, 1094 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.89 (1 H, s, aromatic), 6.82 and 6.73 (2 H, 2 br s, amide protons), 6.00 (1 H, dd, J = 7.5 Hz, J = 3.8 Hz, C(3) proton) 4.30 and 4.80 (2 H, 2 m, C(1) diastereomeric methylene) 3.13 and 2.55 (2 H, 2 m, C(2) diastereomeric methylene), 2.54 (3 H, s, 7-methyl); mass spectrum (EI mode), m/z 354 and 356 (P$^+$, ^{79}Br and P$^+$, ^{81}Br), 311 and 313 (P$^+$ − O=C=NH), 293 and 295 (P$^+$ − carbamic acid). Anal. Calcd for $C_{12}H_{14}BrN_4O_2$: C, 40.58; H, 3.11; N, 15.77. Found: C, 40.61; H, 3.13; N, 15.41.

5-Amino-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Acetate and 3-Carbamate (7c and 7d). A suspension of **6** or **8** in 100 mL of methanol containing 60 mg of 5% Pd on charcoal was shaken under 50 psi of H_2 for 6 h. The reaction was filtered through Celite, and the filter cake was washed with methanol. Acidification of the filtrate with a few drops of 1 N HCl and evaporation in vacuo afforded the dihydrochloride salt of the amine. Recrystallization was carried out from ethyl acetate/methanol.

Reduction of **6** afforded an 80% yield of the dihydrochloride salt of **7c**: mp 250 °C dec; TLC (chloroform/methanol [90:10]) R_f = 0.67; IR (KBr pellet) 3384, 3313, 3205, 2853, 2836, 2752, 1750, 1643, 1494, 1218 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 6.99 (1 H, s, C(8) proton), 6.73 (1 H, s, C(6) proton), 6.25 (1 H, dd, J = 7.9 Hz, J = 3.7 Hz, C(3) proton), 4.40 and 4.26 (2 H, 2 m, C(1) diastereomeric methylene), 3.19 and 2.70 (2 H, 2 m, C(2) diastereomeric methylene), 2.38 (3 H, s, 7-methyl), 2.12 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 245 (P$^+$ of base), 202 (P$^+$ − acetyl), 186 (P$^+$ − acetamide).

Reduction of **8** afforded an 87% yield of the dihydrochloride salt of **7d**: mp 245 °C dec; TLC (chloroform/methanol [80:20]) R_f = 0.48; IR (KBr pellet) 3315, 3270, 3200, 3146, 3041, 1736, 1402, 1370, 1318 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 7.04 and 6.93 (2 H, 2 br s, amide protons), 6.88 (1 H, s, C(8) proton), 6.64 (1 H, s, C(6) proton), 6.12 (1 H, dd, J = 8.0 Hz, J = 3.9 Hz, C(3) proton), 4.39 and 4.24 (2 H, 2 m, C(2) diastereomeric methylene), 3.22 and 2.65 (2 H, 2 m, C(2) diastereomeric methylene), 2.36 (3 H, s, 7-methyl); mass spectrum (EI mode), m/z 246 (P$^+$), 202 (P$^+$ − O=CNH$_2$), 185 (P$^+$ − carbamic acid).

7-Methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Acetate and 3-Carbamate (9c and 9d). To a suspension of 0.35 mmol of **7c** or **7d** in 10 mL of water containing 80 mg of monobasic potassium phosphate was added a solution of 500 mg of Fremy's salt in 50 mL of water containing 200 mg of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 1.5 h and then extracted five times with 20 mL of chloroform. The dried extracts (sodium sulfate) were concentrated to an oil and then flash chromatographed, employing silica gel with acetone (**9d**) or chloroform (**9c**) as eluant. The product was recrystallized from acetone/hexane.

Oxidation of **7c** afforded a 54% yield of **9c**: mp 132–135 °C; TLC (acetone) R_f = 0.67; IR (KBr pellet) 1746, 1739, 1673, 1653, 1610, 1510, 1372, 1329, 1235, 1154 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.54 (1 H, q, J = 1.2 Hz, C(6) proton), 6.09 (1 H, dd, J = 7.6 Hz, J = 2.9 Hz, C(3) proton), 4.40 and 4.31 (2 H, 2 m, C(1) diastereomeric methylene), 3.18 and 2.66 (2 H, 2 m, C(2) diastereomeric methylene), 2.11 (3 H, d, J = 1.2 Hz, 7-methyl), 2.10 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 260 (P^+), 217 (P^+ – acetyl), 200 (P^+ – acetic acid). Anal. Calcd for $C_{13}H_{12}N_2O_4$: C, 59.99; H, 4.64; N, 10.76. Found: C, 59.55; H, 4.70; N, 10.53.

Oxidation of **7d** afforded a 58% yield of **9d**: mp 201 °C dec; TLC (acetone) R_f = 0.53; IR (KBr pellet) 3411, 1741, 1735, 1727, 1654, 1610, 1330, 1168, 1155, 1147 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.54 (1 H, q, J = ~1 Hz, C(6) proton), 6.01 (1 H, dd, J = 7.6 Hz, J = 3.3 Hz, C(3) proton), 4.70 (2 H, br s, amide protons), 4.40 and 4.29 (2 H, 2 m, C(1) diastereomeric methylene) 3.17 and 2.73 (2 H, 2 m, C(2) diastereomeric methylene), 2.10 (3 H, d, J ~1 Hz, 7-methyl); mass spectrum (EI mode), m/z 261 (P^+), 217 (P^+ – O=CNH$_2$), 201 (P^+ – carbamate). Anal. Calcd for $C_{12}H_{11}N_3O_4$: C, 55.17; H, 4.24; N, 16.08. Found: C, 55.65; H, 4.28; N, 16.26.

6-*N*-Aziridinyl-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Acetate (1c). To a solution of 52 mg (0.2 mmol) of **9c** in 2 mL of methanol, chilled at 0 °C, was added 0.5 mL of ethylenimine. After stirring at 0 °C for 30 min, the reaction was stirred at room temperature for 1 h. The solvent was then removed in vacuo, and the brick-red residue was flash chromatographed on silica gel using chloroform as eluant. The purified product was recrystallized from methylene chloride/hexane: 25 mg (42%) yield; mp 125–127 °C; TLC (acetone) R_f = 0.65; IR (KBr pellet) 1746, 1679, 1636, 1518, 1378, 1341, 1314, 1230, 1141, 1035 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.05 (1 H, dd, J = 7.5 Hz, J = 3 Hz, C(3) proton), 4.29 (2 H, m, C(1) diastereomeric methylene), 3.13 and 2.62 (2 H, 2 m, C(2) diastereomeric methylene), 2.36 (4 H, s, aziridine protons), 2.09 (3 H, s, 7-methyl), 2.07 (3 H, s, acetate methyl); ^{13}C NMR ($CDCl_3$) 178.0, 176.7, 169.9, 155.9, 153.1, 144.5, 130.3, 124.6, 66.4, 43.6, 35.0, 29.4, 20.8, 9.5 cps; mass spectrum (EI mode), m/z 301 (P^+), 286 (P^+ – methyl), 258 (P^+ – acetyl). Anal. Calcd for $C_{15}H_{15}N_3O_4$: C, 59.79; H, 5.01; N, 13.94. Found: C, 59.65; H, 5.06; N, 12.96–13.28.

6-*N*-Aziridinyl-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione 3-Carbamate (1d). A solution of 26 mg (0.1 mmol) of **9d** in 7 mL of methanol was combined with 0.25 mL of ethylenimine, and the mixture was stirred at room temperature for 1.5 h. The solvent was evaporated in vacuo, and the red residue was flash chromatographed on silica gel using acetone as eluant. The product was recrystallized from acetone/hexane: 15 mg (50%) yield; mp 185 °C dec; TLC (acetone) R_f = 0.52; IR (KBr pellet) 3444, 3364, 1727, 1653, 1325, 1311 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.98 (1 H, dd, J = 7.5 Hz, J = 3 Hz, C(3) proton), 4.68 (2 H, br s, amide protons), 4.36 and 4.28 (2 H, 2 m, C(1) diastereomeric methylene), 3.15 and 2.71 (2 H, 2 m, C(2) diastereomeric methylene), 2.36 (4 H, s, aziridinyl protons), 2.08 (3 H, s, 7-methyl); ^{13}C NMR ($CDCl_3$) 178.0, 176.7, 156.0, 155.3, 153.0, 144.4, 130.3, 124.5, 67.2, 43.6, 35.1, 29.4, 9.5 cps; mass spectrum (EI mode), m/z 302 (P^+), 259 (P^+ – O=C=NH). Anal. Calcd for $C_{14}H_{14}N_4O_4 \cdot 0.5H_2O$: C, 54.01; H, 4.85; N, 17.99. Found: C, 54.03; H, 4.58; N, 16.81–16.70.

2,4-Dinitro-5-*N*-pyrrolidinotoluene (10). A mixture of 5-bromo-2,4-dinitrotoluene[35] (2.61 g, 10 mmol) and pyrrolidine (2.49 g, 35 mmol) was heated at 90–100 °C for 2 h. The resulting dark brown oil was combined with cracked ice, and the precipitated solids were filtered off, washed with water, and vacuum dried. Purification was carried out by flash chromatography of the solids on a silica gel column using chloroform/hexane (50:50) as eluant. Evaporation of the eluants afforded **10** as orange needles: 2.0 g (82%) yield; mp 142 °C; TLC (chloroform) R_f = 0.82; IR (KBr pellet) 1606, 1566, 1510, 1369, 1350, 1334, 1301, 1276, 1130, 833 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.83 and 8.53 (2 H, 2 s, aromatic protons), 3.27 (4 H, m, methylenes adjacent to pyrrolidine nitrogen), 2.61 (3 H, s, methyl), 1.99 (4 H, m, other pyrrolidine methylenes); mass spectrum (EI mode), m/z 251 (P^+), 234 (P^+ – OH). Anal. Calcd for $C_{11}H_{13}N_3O_4$: C, 52.58; H, 5.21; N, 16.72. Found: C, 52.51; H, 5.27; N, 16.64.

2,4-Diacetamido-5-*N*-pyrrolidinotoluene (11). A suspension of 1.2 g (4.78 mmol) of **10** and 120 mg of 5% Pd on charcoal in 20 mL of methanol was shaken under 50 psi of H_2 for 4 H. The mixture was then filtered through Celite, and the filtrate was combined with 10 mL of acetic anhydride. After this solution was stirred for 1 h, the solvent was removed in vacuo, and ether was added to crystallize the residue. Yield of crude product, suitable for the next step, was 1.05 g (81%). An analytical sample was prepared by recrystallization from chloroform/hexane: mp 234 °C dec; TLC (chloroform/methanol [80:20]) R_f = 0.61; IR (KBr pellet) 3261, 1651, 1616, 1526, 1491, 1464, 1454, 1416, 1368, 1280 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.11 and 9.02 (2 H, 2 s, amide protons), 7.21 and 6.65 (2 H, 2 s, aromatic protons), 3.11 (4 H, m, methylenes adjacent to pyrrolidine nitrogen), 2.09, 1.99, and 1.98 (9 H, 3 s, methyls), 1.84 (4 H, m, other pyrrolidine methylenes); mass spectrum (EI mode), m/z 275 (P^+), 232 (P^+ – acetyl), 217 (P^+ – acetamido). Anal. Calcd for $C_{15}H_{21}N_3O_2$: C, 65.42; H, 7.68; N, 15.26. Found: C, 65.00; H, 7.68; N, 15.00.

6-Acetamido-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (13). A mixture of 1 g (3.63 mmol) of **11**, 6 mL of 96% formic acid, and 3 mL of 30% hydrogen peroxide was stirred at 70 °C for 30 min. The reaction mixture color changed from blue to red-brown and finally to yellow upon completion. The reaction mixture was then diluted with water and neutralized to pH 7.00 with concentrated ammonium hydroxide. Extraction of the neutralized solution with 2 × 50-mL portions of chloroform, drying the extracts (sodium sulfate), and concentration afforded crude **13** as a yellow solid. Recrystallization was carried out from chloroform/hexane: 676 mg (81%) yield; mp 200 °C dec; TLC (chloroform/methanol [90:10]) R_f = 0.42; IR (KBr pellet) 3442, 3230, 1668, 1526, 1476, 1456, 1423, 1309, 1304, 1283 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.23 (1 H, s, amide proton), 7.45 and 7.23 (2 H, 2 s, aromatic protons), 4.04 (2 H, t, J ~ 7 Hz, C(1) methylene), 2.91 (2 H, t, J ~ 7 Hz, C(3) methylene), 2.61 (2 H, quintet, J ~ 7 Hz, C(2) methylene), 2.26 (3 H, s, 7-methyl), 2.04 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 229 (P^+), 187 (P^+ – ketene). Anal. Calcd for $C_{13}H_{15}N_3O \cdot 0.5H_2O$: C, 65.47; H, 6.76; N, 17.62. Found: C, 65.90; H, 6.59; N, 17.75.

7-Methyl-6-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Acetate (12). A mixture consisting of 2.5 g (10 mmol) of **10**, 2.72 g (20 mmol) of $ZnCl_2$, and 10 mL of acetic anhydride was refluxed (90–100 °C) for 4 h. The reaction mixture was then cooled and combined with 100 mL of water. Extraction of the diluted reaction mixture with 3 × 50-mL portions of chloroform and concentration of the dried (sodium sulfate) extracts afforded crude product. Purification by silica gel chromatography, using ethyl acetate/methanol (95:5) as eluant, afforded pure **12** as a light yellow powder: 1.4 g (53%) yield; mp 172 °C dec; TLC (chloroform/methanol [90:10]) R_f = 0.51; IR (KBr pellet) 1738, 1527, 1373, 1344, 1318, 1297, 1261, 1248, 1078, 1034 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.48 (1 H, s, C(5) aromatic), 7.27 (1 H, s, C(8) aromatic), 6.20 (1 H, dd, J = 7.6 Hz, J = 3.8 Hz, C(3) proton), 4.31 and 4.17 (2 H, 2 m, C(1) diastereomeric methylene), 3.24 and 2.72 (2 H, 2 m, C(2) diastereomeric methylene), 2.72 (3 H, s, 7-methyl), 2.15 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 275 (P^+), 258 (P^+ – OH), 232 (P^+ – acetyl). Anal. Calcd for $C_{13}H_{13}N_3O_4$: C, 56.67; H, 4.72; N, 15.27. Found: C, 56.61; H, 4.72; N, 14.97.

6-Acetamido-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Acetate (14). A solution of 1.1 g (3.99 mmol) of **12** in 200 mL of methanol was shaken under 50 psi of H_2 in the presence of 200 mg of 5% Pd on carbon for 4 h. The completed reaction was filtered through Celite into a flask containing 2 mL of acetic acid. The filtrate was then evaporated in vacuo to an acetic acid/amine mixture, to which was added 6 mL of acetic anhydride. This mixture was stirred for 30 min at room temperature and then diluted with 200 mL of diethyl ether. Pure **14** crystallized from the ether solution after chilling for several hours: 809 mg (70%) yield. Recrystallization was carried out from

(35) Gitis, S. S.; Trunov-Krasovskii, V. I. *Zhur. Obshchei. Khim.* **1959**, *29*, 2646; *Chem. Abstr.* **1960**, *54*, 10924*h*.

a large volume of hot ethyl acetate: mp 232 °C dec; TLC (1-butanol–acetic acid–water [5:2:3]) R_f = 0.4; IR (KBr pellet) 3260, 1741, 1647, 1537, 1368, 1233, 1145, 1131 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.28 (1 H, s, amide proton), 7.57 and 7.37 (2 H, 2 s, aromatic protons), 6.10 (1 H, dd, J = 7.6 Hz, J = 3.2 Hz, C(3) proton), 4.22 and 4.12 (2 H, 2 m, C(1) diastereomeric methylene), 3.11 and 2.55 (2 H, 2 m, C(2) diastereomeric methylene), 2.29 (3 H, s, 7-methyl), 2.07 and 2.05 (6 H, 2 s, acetate and acetamido methyls).

6-Acetamido-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (15a). To a mixture of 5.4 mL of fuming nitric acid and 0.6 mL of concentrated sulfuric acid, chilled at 0 °C, was added 600 mg (2.61 mmol) of **13**. The reaction mixture was stirred at 0 °C for 5 min and then poured into a mixture of 20 g of cracked ice and 30 mL of chloroform. The mixture was neutralized with saturated aqueous sodium bicarbonate and vigorously stirred to extract the product into the chloroform layer. The chloroform layer was removed, and the aqueous layer was extracted with 3 × 30-mL portions of chloroform. Drying the combined chloroform extracts (sodium sulfate) and concentration afforded **15a** as a yellow solid. Recrystallization was carried out from chloroform/hexane: 500 mg (69%) yield; mp 198 °C; TLC (chloroform/methanol [85:15]) R_f = 0.44; IR (KBr pellet) 1689, 1525, 1517, 1492, 1459, 1421, 1369, 1358, 1263, 1249 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.89 (1 H, s, amide proton), 7.38 (1 H, s, C(8) proton), 4.14 (2 H, t, J = 7.4 Hz, C(1) methylene), 3.14 (2 H, t, J = 7.4 Hz, C(3) methylene), 2.78 (2 H, quintet, J = 7.4 Hz, C(2) methylene), 2.41 (3 H, s, 7-methyl), 2.21 (3 H, s, acetamido methyl); mass spectrum (EI mode), m/z 274 (P$^+$), 256 (P$^+$ – H$_2$O), 232 (P$^+$ – ketene), 228 (P$^+$ – NO$_2$). Anal. Calcd for $C_{13}H_{14}N_4O_3{\cdot}1.5H_2O$: C, 51.84; H, 4.68; N, 18.59. Found: C, 51.64; H, 4.68; N, 18.52.

6-Acetamido-7-methyl-5-nitro-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole 3-Acetate (15b). To a mixture of 2 mL of fuming nitric acid and 0.8 mL of concentrated sulfuric acid, chilled in a dry ice–acetone bath, was added 400 mg (1.39 mmol) of **14** portionwise over a 2-min period. The reaction mixture was removed from the ice bath and stirred for 15 min while coming to room temperature and then poured into a mixture of 50 g of ice and 50 mL of chloroform. Saturated sodium bicarbonate was added to the above mixture with vigorous stirring until the pH was neutral. The chloroform layer was separated, and the aqueous layer was extracted twice with 50-mL portions of chloroform. Drying the combined extracts (sodium sulfate), concentration to a residue, and trituration with ethyl acetate afforded crystalline **15b**: 310 mg (67%) yield. Recrystallization was carried out from chloroform/hexane: mp 204 °C; TLC (chloroform/methanol [9:1]) R_f = 0.24; IR (KBr pellet) 1750, 1681, 1528, 1370, 1360, 1270, 1083 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.89 (1 H, s, amide proton), 7.47 (1 H, s, C(8) proton), 6.17 (1 H, dd, J = 7.5 Hz, J = 3.5 Hz, C(3) proton), 4.2 (2 H, m, C(1) diasteromeric methylene), 3.17 and 2.72 (2 H, 2 m, C(2) diastereomeric methylene), 2.43 (3 H, s, 7-methyl) 2.22 and 2.13 (6 H, 2 s, acetate and acetamido protons); mass spectrum (EI mode), m/z 332 (P$^+$), 314 (P$^+$ – H$_2$O), 286 (P$^+$ – NO$_2$). Anal. Calcd for $C_{15}H_{16}N_4O_5{\cdot}0.25H_2O$: C, 53.49; H, 4.93; N, 16.62. Found: C, 53.78; H, 4.62; N, 16.42.

6-Acetamido-5-amino-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (16a) and the 3-Acetate Derivative (16b). A solution of 1.2 mmol of **15a** or **15b** in 60 mL of methanol was shaken under 50 psi of H$_2$ in the presence of 40 mg of 5% Pd on carbon for 2.5 h. The catalyst was removed by filtration through Celite, and the filtrate was concentrated to a yellow oil. Dissolution of the oil in 15 mL of chloroform, addition of hexane until the solution became cloudy, and then chilling afforded the amine as a white crystalline solid.

Reduction of **15a** afforded **16a** in 74% yield: mp 235 °C dec; TLC (chloroform/methanol [8:2]) R_f = 0.58; IR (KBr pellet) 3362, 3216, 3209, 1669, 1634, 1552, 1533, 1305, 1297, 1277 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.10 (1 H, s, amide proton), 6.76 (1 H, s, C(8) proton), 4.23 (2 H, t, J = 7.2 Hz, C(1) methylene), 3.28 (2 H, t, J = 7.6 Hz, C(3) methylene), 2.74 (2 H, quintet, $J \sim$ 7.4 Hz, C(2) methylene), 2.18 (3 H, s, 7-methyl), 2.03 (3 H, s, acetate methyl); mass spectrum (EI mode), m/z 244 (P$^+$), 229 (P$^+$ – methyl), 201 (P$^+$ – acetyl). Anal. Calcd for $C_{13}H_{16}N_4O{\cdot}0.6H_2O$: C, 61.21; H, 6.79; N, 21.95. Found: C, 61.13; H, 6.13; N, 21.49.

Reduction of **15b** afforded **16b** in 77% yield: mp 211 °C dec; TLC (chloroform/methanol [80:20]) R_f = 0.48; IR (KBr pellet) 3440, 3399, 1738, 1663, 1620, 1491, 1235 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 8.89 (1 H, s, amide proton), 6.64 (1 H, s, C(8) proton), 6.08 (1 H, dd, J = 7.5 Hz, J = 3 Hz, C(3) proton), 4.96 (2 H, br s, amine protons), 4.14 and 4.05 (2 H, 2 m, C(1) diastereomeric methylene), 3.1 and 2.5 (2 H, 2 m, C(2) diastereomeric methylene), 2.16 (3 H, s, 7-methyl), 2.06 and 2.04 (6 H, 2 s, acetate and acetamido methyls); mass spectrum (EI mode), m/z 302 (P$^+$). Anal. Calcd for $C_{15}H_{18}N_4O_3{\cdot}0.5H_2O$: C, 58.30; H, 6.15; N, 17.98. Found: C, 58.61; H, 5.78; N, 17.76.

6-Acetamido-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole-5,8-dione (1a) and the 3-Acetate Derivative (1b). To a suspension of **16a** or **16b** (0.7 mmol) in 10 mL of water, containing 200 mg of potassium phosphate monobasic, was added a solution of 1 g of Fremy's salt in 30 mL of water containing 500 mg of potassium phosphate monobasic. The mixture was stirred at room temperature for 2.5 h and then extracted three times with 100-mL portions of chloroform. The dried extracts (sodium sulfate) were concentrated to a yellow solid, which was recrystallized from chloroform/hexane.

Oxidation of **16a** afforded **1a** in 71% yield: mp 194 °C dec; TLC (acetone) R_f = 0.41; IR (KBr pellet) 2860, 1651, 1539, 1518, 1485, 1466, 1310, 1279, 1245, 1099 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.70 (1 H, br s, amide proton), 4.24 (2 H, t, J = 7.0 Hz, C(1) methylene), 2.84 (4 H, m, C(2) and C(3) methylenes), 2.24 (3 H, s, 7-methyl), 1.96 (3 H, s, acetamido methyl); ^{13}C NMR (CDCl$_3$) 178.0, 177.7, 167.6, 160.9, 143.9, 135.9, 131.1, 130.6, 45.2, 26.5, 24.2, 22.8, 13.5 cps; mass spectrum (EI mode), m/z 259 (P$^+$), 244 (P$^+$ – methyl), 217 (P$^+$ – ketene). Anal. Calcd for $C_{13}H_{13}N_3O_3$: C, 60.22; H, 5.05; N, 16.20. Found: C, 60.04; H, 4.98; N, 15.93.

Oxidation of **16b** afforded **1b** in 27% yield: mp 221 °C dec; TLC (acetone) R_f = 0.56; IR (KBr pellet) 1730, 1695, 1659, 1610, 1520, 1371, 1314, 1284, 1244, 1083 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.69 (1 H, br s, amide proton), 6.09 (1 H, dd, J = 7.7 Hz, J = 3.3 Hz, C(3)-proton), 4.37 (2 H, m, C(1) diastereomeric methylene), 3.18 and 2.72 (2 H, 2 m, C(2) diastereomeric methylene), 2.25 (3 H, s, 7-methyl), 2.10 and 1.98 (6 H, 2 s, acetate and acetamido methyls); ^{13}C NMR (dimethyl sulfoxide-d_6) 177.3, 176.6, 169.5, 167.9, 156.8, 143.9, 138, 133.5, 129.9, 66.3, 43.5, 34.0, 22.9, 20.5, 12.2 cps; mass spectrum (EI mode), m/z 317 (P$^+$), 300 (P$^+$ – OH), 275 (P$^+$ – ketene). Anal. Calcd for $C_{15}H_{15}N_3O_5$: C, 56.78; H, 4.76; N, 13.27. Found: C, 56.59; H, 4.67; N, 12.87.

***syn*/*anti*-6-Acetamido-5-imino-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-8-one (2a).** To a suspension of 100 mg (0.4 mmol) of **16a** in 10 mL of 0.2 M pH 7.0 phosphate buffer (μ = 1.0, KCl) was added a suspension of 500 mg of Fremy's salt in 20 mL of the same buffer. To assist in dissolution of the Fremy salt, 20 mL of water was then added to the above mixture. While the mixture was stirred at room temperature, purple *syn*-**2a** crystallized from solution. After 30 min, the *syn*-**2a** was filtered off and dried: 69 mg (65%) yield. The filtrate was extracted with 2 × 50 mL of chloroform to remove the anti isomer. Drying the extracts (sodium sulfate), evaporation to a solid residue, and finally recrystallization from chloroform/hexane afforded 10 mg (9.5%) of yellow *anti*-**2a**. Extensive purification of either isomer was not possible due to *syn*/*anti* introconversion in many solvents.

Physical properties of *syn*-**2a**: mp 260 °C dec; TLC (chloroform/methanol [90:10]) R_f = 0.44; IR (KBr) 3250, 1652, 1625, 1608, 1422, 1393 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 9.19 and 6.62 (2 H, 2 br s, imine protons, see Scheme III), 4.12 (2 H, m, C(1) methylene), 2.81 and 2.58 (4 H, 2 m, C(2) and C(3) methylenes), 1.72 and 1.58 (6 H, 2 s, 7-methyl and acetamido methyls); ^{13}C NMR (dimethyl sulfoxide-d_6) 176.5; 158.6, 154.3, 149.5, 138.8, 129.8, 110.4, 96.2, 44.5, 26.1, 25.7, 22.2, 8.7 cps; mass spectrum (EI mode), m/z 258 (P$^+$), 243 (P$^+$ – methyl), 229 (P$^+$ – C=NH), 215 (P$^+$ – acetyl). Anal. Calcd for $C_{13}H_{14}N_4O_2{\cdot}1.25H_2O$: C, 55.60; H, 5.69; N, 19.95. Found: C, 55.35; H, 5.12; N, 19.07.

Physical properties of *anti*-**2a**: mp 245 °C dec; TLC, same as *syn*-**2a**; IR (KBr pellet) 3260, 3200, 1683, 1644, 1625, 1504, 1484, 1465, 1422, 1341, 1314, 1252 cm^{-1}; ^{1}H NMR (dimethyl sulfoxide-d_6) δ 11.42 (1 H, s, imine proton), 9.57 (1 H, s, amide proton), 4.19 (2 H, t, J = 6.8 Hz, C(1) methylene), 2.73 (4 H, m, C(2) and C(3) methylenes), 2.07 and 1.81 (6 H, 2 s, 7-methyl and acetamido methyl); mass spectrum (same as *syn*-**2a**).

***syn*/*anti*-6-Acetamido-5-imino-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazol-8-one 3-Acetate (2b).** To a solution of **16b**, 150 mg (0.49 mmol), in 25 mL of 0.2 M pH 7.0

phosphate buffer (μ = 1.0, KCl) was added 708 mg of Fremy's salt. The mixture was stirred at room temperature for 1 h, during which time red *syn*-**2b** crystallized from solution. Filtration, washing the solids with a small volume of water, and then drying afforded *syn*-**2b** as a fiberous red solid: 61 mg (36%) yield. The filtrate was extracted with 2 × 50 mL of chloroform. Evaporation of the dried extracts ($MgSO_4$) to a residue and then trituration with acetone afforded yellow *anti*-**2b** (21 mg (12%) yield).

Physical properties of *syn*-**2b**: mp 312 °C dec; TLC (acetone) R_f = 0.57; IR (KBr pellet) 3340, 1745, 1625, 1601, 1380, 1238 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 9.34 and 6.68 (2 H, 2 br s, imine protons), 5.99 (1 H, m, C(3) proton), 4.24 (2 H, m, C(1) diastereomeric methylene), 3.04 and ~2.5 (2 H, 2 m, C(2) diastereomeric methylene), 2.07 (3 H, s, 7-methyl), 1.74 and 1.54 (6 H, 2 s, acetate and acetamido methyls); ^{13}C NMR (dimethyl sulfoxide-d_6) 176.2, 169.6, 154.7, 154.1, 149.8, 138.7, 130, 110.6, 96.8, 65.5, 43.3, 34.1, 25.5, 20.6, 8.7 cps; mass spectrum (EI mode), m/z 316 (P^+). Anal. Calcd for $C_{15}H_{16}N_4O_4$·$0.25H_2O$: C, 56.15; H, 5.18; N, 17.45. Found: C, 55.94; H, 5.19; N, 17.18.

Physical properties of *anti*-**2b**: mp 304 °C dec; TLC (same as *syn*-**2b**); IR (KBr pellet) 3188, 1740, 1714, 1644, 1627, 1487, 1376, 1310, 1230 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 11.65 (1 H, s, amide proton), 9.64 (1 H, s, imine proton), 6.06 (1 H, dd, J = 8 Hz, J = 3.8 Hz, C(3) proton), 4.29 (2 H, m, C(1) diastereomeric methylene), 3.06 and ~2.6 (2 H, 2 m, C(2) diastereomeric methylene), 2.08 (6 H, 2 s), and 1.8 (3 H, s), 7-methyl, acetamido, and acetate methyls, no assignments made; mass spectrum (same as *syn*-**2a**).

6-Acetamido-5-amino-8-hydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (19). A solution of 25 mg (0.09 mmol) of *syn*-**2a** in 5 mL of methanol was shaken under 50 psi of H_2 in the presence of 5 mg of 5% Pd on charcoal. The catalyst was then removed by filtering through Celite, and the filtrate immediately concentrated to a solid. Dissolution of the solid in 5 mL of chloroform/methanol (1:4) and adding hexane resulted in precipitation of **19**: 20 mg (79%) yield; TLC (chloroform/methanol [6:4]) R_f = 0.4; IR (KBr pellet) 3322, 3210, 3140, 1660, 1640, 1505 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 9.19 (1 H, s, amide proton), 8.68 (1 H, s, 8-hydroxyl), 4.14 (2 H, t, J = 7.1 Hz, C(1) methylene), 3.20 (2 H, t, J = 7.5 Hz, C(3) methylene), 2.72 (2 H, m, C(2) methylene), 2.07 and 2.04 (6 H, 2 s, 7-methyl and acetamido methyl); mass spectrum (EI mode), m/z 260 (P^+), 242 ($P^+ - H_2O$), 217 (P^+ − acetyl).

6-Acetamido-5,6-dihydroxy-7-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]benzimidazole (20). A solution of 30 mg (0.11 mmol) of **1a** in 10 mL of methanol was shaken under 50 psi of H_2 for 25 min in the presence of 8 mg of 5% Pd on charcoal. After addition of 3 drops of concentrated HCl to the reaction, the catalyst was removed by filtering through Celite, and the filtrate was concentrated to a solid. Recrystallization of the solid by dissolution in a minimal amount of methanol followed by addition of ethyl acetate afford **20** as the HCl salt: 32 mg (97%) yield; TLC (chloroform/methanol [6:4]) R_f = 0.57; IR (KBr pellet) 3337, 3150, 1650, 1505, 1299 cm^{-1}; ^1H NMR (dimethyl sulfoxide-d_6) δ 9.39 (2 H, s, 5,8-dihydroxy), 9.03 (1 H, s, amide proton), 4.45 (2 H, t, J = 6.9 Hz, C(1) methylene), 3.22 (2 H, t, J = 7.5 Hz, C(3) methylene), 2.72 (2 H, quintet, J = 7.6 Hz, C(2) methylene), 2.10 and 2.08 (6 H, 2 s, 7-methyl and acetamido methyl); mass spectrum (EI mode), m/z 261 (P^+), 243 ($P^+ - H_2O$), 219 (P^+ − ketene).

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Registry No. **1a**, 123567-03-3; **1b**, 123567-28-2; **1c**, 123567-24-8; **1d**, 123567-25-9; *syn*-**2a**, 123592-95-0; *anti*-**2a**, 123567-29-3; *syn*-**2b**, 123593-07-7; *anti*-**2b**, 123567-30-6; **3**, 123567-04-4; **4**, 123567-05-5; 4 deacetylated derivative, 123567-31-7; **5**, 123567-06-6; **6**, 123567-07-7; **6** deacetylated derivative, 123567-20-4; **6** phenyl carbonate analogue, 123567-21-5; **7c**·2HCl, 123567-08-8; **7d**·2HCl, 123567-22-6; **8**, 123567-09-9; **9c**, 123567-10-2; **9d**, 123567-23-7; **10**, 123567-11-3; **11**, 123567-12-4; **12**, 123567-13-5; **13**, 123567-14-6; 14, 123567-15-7; **15a**, 123567-16-8; **15b**, 123567-26-0; **16a**, 123567-17-9; **16b**, 123567-27-1; **19**, 123567-18-0; **20**·HCl, 123567-19-1; 3-bromo-4-nitrotoluene, 40385-54-4; pyrrolidine, 123-75-1; ethylenimine, 151-56-4; 5-bromo-2,4-dinitrotoluene, 5411-53-0.

A Novel and Versatile Synthesis of 1-Alkyl-, 1-Aryl-, 1-(Alkylamino)-, or 1-Amido-Substituted and of 1,2,6-Trisubstituted Piperidines from Glutaraldehyde and Primary Amines or Monosubstituted Hydrazines[1]

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Various primary amines and 1-mono- and 1,1-disubstituted hydrazines were converted into the corresponding N-substituted piperidines in good to excellent yields via the products of double condensations with benzotriazole and glutaraldehyde. Reduction of the 2,6-bis(benzotriazolyl) N-substituted piperidines 4 and 7 with sodium borohydride in tetrahydrofuran afforded N-substituted piperidines. The benzotriazole moieties were also replaced by alkyl groups by reaction with Grignard reagents to produce 1,2,6-trisubstituted piperidines.

Many N-substituted piperidines and their 2,6-dialkyl derivatives are pharmacologically active and form an essential part of the molecular structure for important drugs.[2] For example, the 1-piperidino group is a feature of the antihistaminic agent and the spasmolytic benzhexol,[3] of narcotic analgesics,[4] of postganglionic parasympathetic agonists,[5] and of oral anesthetics.[6] Many 1,2,6-trialkylpiperidine alkaloids have been isolated from both animal and plant species.[7,8]

(1) The Chemistry of Benzotriazole. See: Katritzky, A. R.; Urogdi, L.; Mayence, A. *J. Chem. Soc., Chem. Commun.* **1989**, 337. Katritzky, A. R.; Hughes, C. V. *Chem. Scr.* **1989**, *29*, 27, and refs 24–28.

(2) Coutts, R. T.; Casy, A. F. In *Pyridine and its Derivatives*; Abramovitch, R. A., Ed.; Interscience: John Wiley & Sons: New York, 1974; Part 4, P 492.

(3) Casy, A. F.; Ison, R. R. *J. Pharm. Pharmacol.* **1970**, *22*, 270.

(4) Reynolds, A. K.; Randall, L. O. *Morphine and Allied Drugs*; University of Toronto Press: Toronto, 1957; p 269.

(5) Hermons, B.; van Daele, P.; van de Westeringh, C.; van der Eycken, C.; Boey, J.; Dockx, J.; Janssen, P. A. J. *J. Med. Chem.* **1968**, *11*, 797.

(6) McElvain, S. M. *J. Am. Chem. Soc.* **1927**, *49*, 2835.

(7) MacConnel, J. G.; Blum, M. S.; Fales, H. M. *Tetrahedron*, **1971**, *27*, 1129.

(8) Hootele, C.; Colau, B.; Halin, F. *Tetrahedron Lett.* **1980**, *21*, 5063.

0022-3263/90/1955-3205$02.50/0