Studies of Extended Quinone Methides. Synthesis and Physical Studies of Purine-like Monofunctional and Bifunctional Imidazo[4,5-g]quinazoline Reductive Alkylating Agents

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Described herein are the synthesis, quinone methide reactivity, and electrochemistry of purine-like imidazo[4,5-g]quinazoline reductive alkylating agents possessing a leaving group at the 6α-position. Also described is the synthesis of a dual-alkylating analogue possessing a leaving group at both the 2α- and 6α-positions. The reductive alkylating agent design involves leaving group placement on the 4,9-dione (quinone) derivative of the title ring system so as to permit formation of an alkylating quinone methide species upon reduction to the hydroquinone and elimination of the leaving group. The purine-like structure of these reductive alkylating agents may permit selective inactivation of purine-utilizing enzymes in low reduction potential tumor cells. Comparisons of our finding with those obtained for an analogous reductive alkylation system revealed the following: (i) lowering the quinone reduction potential greatly enhances the rate of leaving group elimination (e.g., a 2300-fold increase in the rate of chloride elimination accompanies a 200-mV potential decrease), and (ii) lower potentials favor electrophile trapping (ketonization) over nucleophile trapping of the quinone methide intermediate. The results of our studies indicate electrochemical studies are valuable in predicting the reactivity pattern of a reductive alkylating agent.

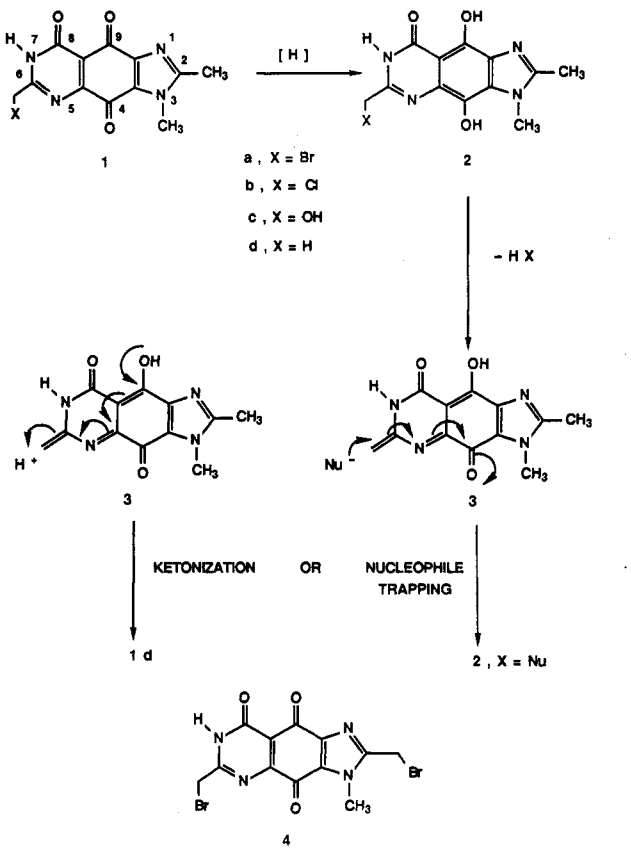
Previous studies in this laboratory have shown that purine-like reductive alkylating agents can be designed by functionalizing the imidazo[4,5-g]quinazoline ring system as a quinone (4,9-dione) with a leaving group placed at the 2α-position.[2,3] Upon reduction to the hydroquinone form, the leaving group is eliminated to afford an alkylating quinone methide species. This same sequence of events is thought to occur in many naturally occurring quinone reductive alkylating systems.[4] Mitomycin C, for example, crosslinks DNA specifically in the low reduction potential environment of tumor cells.[5] The possible utility of purine-like reductive alkylating agents is their selective inactivation of purine-utilizing enzymes in this low reduction potential environment.

Presented herein are the synthesis and physical studies of imidazo[4,5-g]quinazoline reductive alkylating agents possessing a leaving group at the 6α-position (**1a–c**) as well as the synthesis of an analogue possessing a leaving group at both the 2α- and 6α-position (**4**) (Scheme I).

Kinetic studies of the hydrolysis of **2b** in anaerobic buffers indicate the steady-state presence of a quinone methide species **3** capable of both trapping nucleophiles and ketonizing to a quinone (Scheme I). Electrochemical studies indicate that the reductive alkylating agents in Scheme I possess 200 mV lower reduction potentials than the quinazoline analogues.[6] As a result of the electron-rich character of imidazo[4,5-g]quinazolines, relative to quinazolines, elimination of chloride from **2b** is ~2300 times faster than from the quinazoline analogue, and ketonization of **3** is thermodynamically favored over nucleophile trapping. We conclude that electrochemical studies are valuable in predicting the rate of formation and fate of a quinone methide intermediate.

Synthetic studies described here and elsewhere[2,3] have resulted in a series of purine-like reductive alkylating agents possessing leaving groups at various positions on the imidazo[4,5-g]quinazoline ring (2α, 6α, and both 2α and 6α). The availability of these agents will permit a nucleophile search in the active site in enzymes tolerating a dimensional change in purine substrates.[7]

Scheme I



Results and Discussion

Synthesis. The synthesis of the quinones **1** and **4** is discussed below in conjunction with Scheme II. Previous studies have shown that the imidazo[4,5-g]quinazoline

(1) National Institutes of Health research career development award recipient 1988–1993 (CA01349).
(2) Lee, C.-H.; Gilchrist, J. H.; Skibo, E. B. *J. Org. Chem.* **1986**, *51*, 4787.
(3) Lee, C.-H.; Skibo, E. B. *Biochemistry* **1987**, *26*, 7355.
(4) Moore, H. W.; Czerniak, R. *Med. Res. Rev.* **1981**, *1*, 249.
(5) Kennedy, K. A.; Rockwell, S.; Sartorelli, A. C. *Cancer Res.* **1980**, *40*, 2356.
(6) Lemus, R. L.; Skibo, E. B. *J. Org. Chem.* **1988**, *53*, 6099.
(7) (a) Leonard, N. J. *Acc. Chem. Res.* **1982**, *15*, 128. (b) Leonard, N. J.; Hiremath, S. P. *Tetrahedron* **1986**, *42*, 1917.

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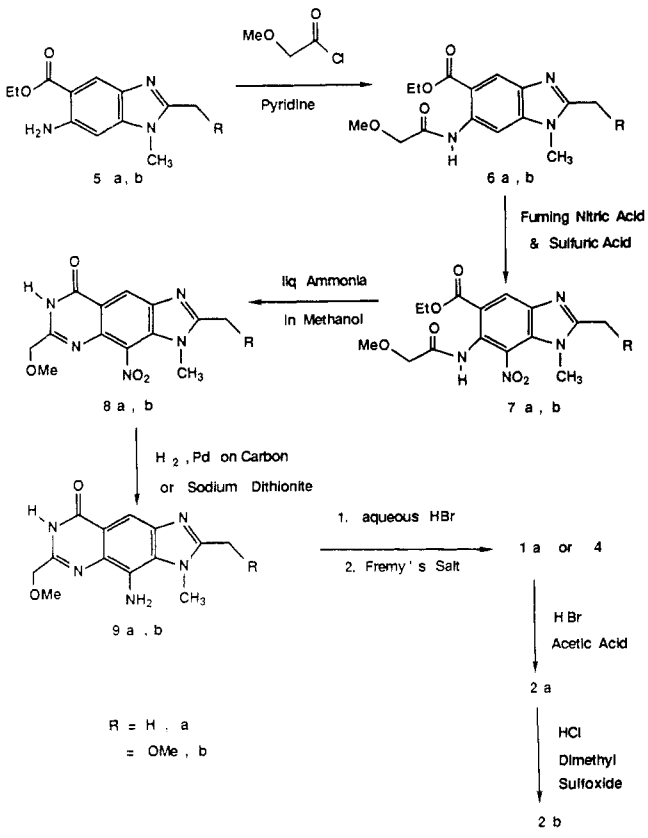
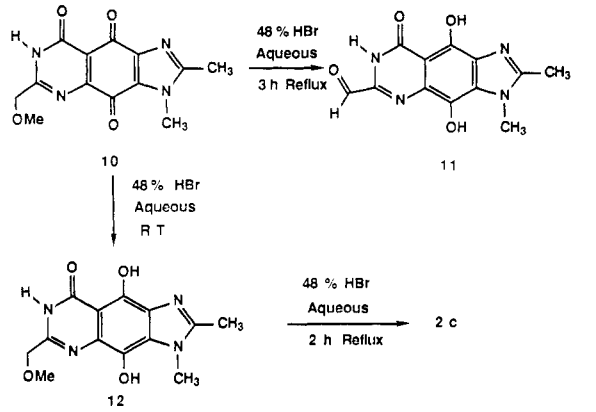
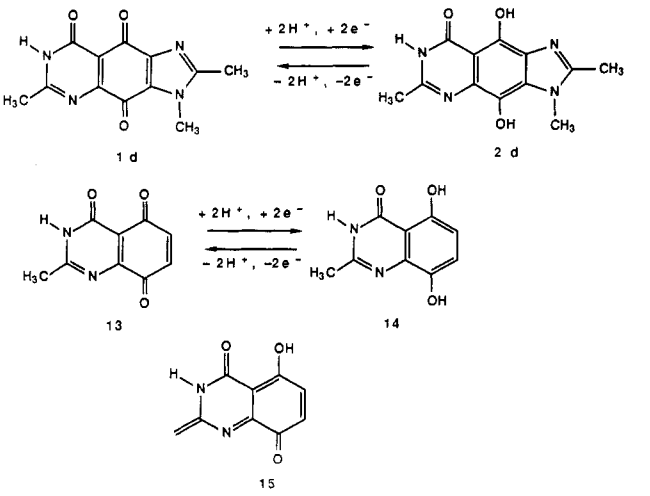
Scheme II



Scheme III



Scheme IV



system is not easily nitrated.[2] Thus to introduce a nitro group for later elaboration of the quinone functionality, nitration of benzimidazoles **6** to afford **7** was carried out before annelation of the pyrimidine ring. The presence of the nitro group in **7** facilitates the conversion to imidazo[4,5-*g*]quinazolines **8** due to electron withdrawal from the α-methoxyacetamido group.[8] The bromo group(s) was introduced by treatment of **9** with aqueous 48% aqueous HBr. Oxidation of the bromomethyl analogues of **9** with Fremy's salt afforded the desired quinones **1a** and **4**. Treatment of **1a** with HBr/acetic acid afforded hydroquinone **2a** with concomitant formation of bromine.[2] Conversion of **2a** to the α-chloro analogue **2b** was carried out by treatment with concentrated HCl in dimethyl sulfoxide.

The results of our previous synthetic efforts[2] suggested that **2a** could also be prepared by treating **10** with a refluxing aqueous 48% HBr. Thus, the HBr would reduce quinone to hydroquinone as well as convert methoxymethyl to bromomethyl. The reduction reaction (**10** → **12**) does occur upon treatment of **10** with aqueous 48% HBr at room temperature (Scheme III). However, treatment of **10** with refluxing aqueous 48% HBr afforded the aldehyde **11**. The production of **11** probably involves the following: formation of **12** and bromine, the radical bromination of **12** at the 6α-position, and hydrolysis of the resulting 6α-bromo, 6α-methoxy derivative. Bromine is necessary for aldehyde formation since the treatment of isolated **12** with HBr affords only the 6α-hydroxy derivative **2c**. Consistent with the postulated mechanism for **11** formation, radical halogenation has been observed at the α-position of methylated pyrimidine derivatives.[9] In contrast, 2-methylbenzimidazole derivatives do not undergo α-halogenation,[10] and consequently aldehyde formation is not observed when the 2α-methoxy isomer of **10** is treated with refluxing HBr.[2]

Electrochemistry. Previous studies in this laboratory suggested that the fate of a quinone methide (ketonization vs nucleophile trapping, Scheme I) can be predicted from the reduction potential of the quinone arising from ketonization.[6] The quinone reduction potential is related to the relative thermodynamic stability of the quinone and hydroquinone products arising from the quinone methide. Thus, a low reduction potential ketonization product would result in a predominance of quinone methide ketonization over nucleophile trapping. The opposite would be true if a high reduction potential quinone results from quinone methide ketonization. The potential of the reductive alkylation system would also influence leaving group elimination from the hydroquinone form; a decrease in reduction potential would increase the electron density of the system and thereby increase the rate of leaving group elimination.

To test the above predictions, we have made a comparison between the previously studied[6] quinazoline-based quinone methide (**15**) and the structurally related imidazo[4,5-*g*]quinazoline quinone methide (**3**) reported herein. Thermodynamic information needed for this comparison was obtained from a Nernst fit for the redox couple arising from ketonization of **3**, **1d**/**2d** in Scheme IV. The Nernst fit for the ketonization product of **15**, **13**/**14** in Scheme IV, is reproduced from the previous study to illustrate the electronic influence of the fused 2,3-dimethylimidazole ring. The results of these studies correctly predicted that

(8) Skibo, E. B.; Gilchrist, J. H. *J. Org. Chem.* **1988**, *53*, 4209.
(9) Cohen, S.; Thom, E.; Bendich, A. *J. Org. Chem.* **1962**, *27*, 3545.
(10) Holan, G.; Samuel, E. L.; Ennis, B. C.; Hinde, R. W. *J. Chem. Soc. C* **1967**, 20.

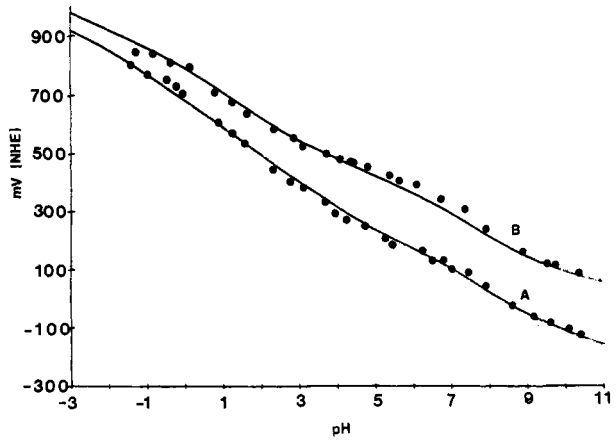


Figure 1. Em vs pH data for the two-electron couples **1d/2d** (plot A) and **13/14** (plot B) measured at 25–26 °C in anaerobic buffer (μ = 1.0, $NaClO_4$). The solid curves were generated employing the Nernst equation.

Table I. Quinone and Hydroquinone pK_a Values for the Couple 1d/2d

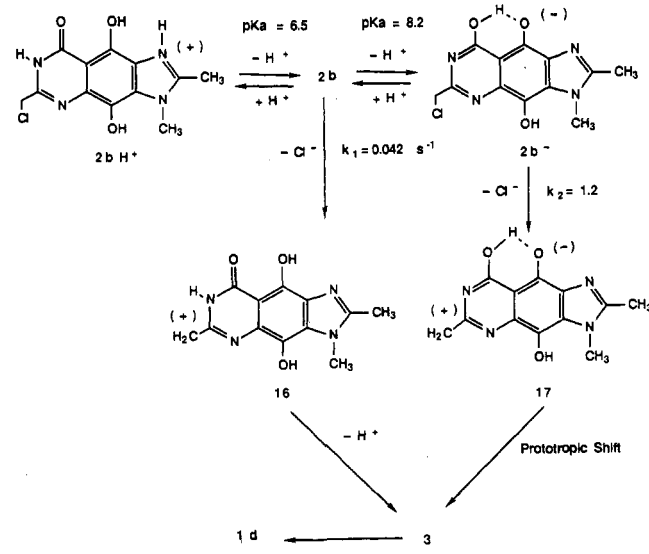
entry	acid dissociation	acid species	pK_a
1	$\mathbf{1dH_2^{2+}} \rightleftarrows \mathbf{1dH^+} + H^+$	N(1),N(5)-diprotonated quinone	~−2
2	$\mathbf{1dH^+} \rightleftarrows \mathbf{1d} + H^+$	N(1)-protonated quinone	1.59 ± 0.03
3	$\mathbf{1d} \rightleftarrows \mathbf{1d^-} + H^+$	N(7)-proton of quinone	6.89 ± 0.02
4	$\mathbf{2dH_2^{2+}} \rightleftarrows \mathbf{2dH^+} + H^+$	N(1),N(5)-diprotonated hydroquinone	2.10 ± 0.07
5	$\mathbf{2dH^+} \rightleftarrows \mathbf{2d} + H^+$	N(1)-protonated hydroquinone	~4.7
6	$\mathbf{2d} \rightleftarrows \mathbf{2d^-} + H^+$	9-OH of hydroquinone	8.54 ± 0.04
7	$\mathbf{2d^-} \rightleftarrows \mathbf{2d^{2-}} + H^+$	4-OH of hydroquinone	~10.5
8	$\mathbf{2d^{2-}} \rightleftarrows \mathbf{2d^{3-}} + H^+$	N(7)-H of hydroquinone	>14

3 ketonizes as well as forms much more readily than **15**.

Electrochemical redox potentials for **1d/2d** were determined, as a function of pH, with conventional cyclic voltammetry employing a glassy carbon electrode. The measurements were made in anaerobic μ = 1.0 ($NaClO_4$) aqueous buffer over the pH range −1.3 to 10.3 at 25–26 °C. The cyclic voltammograms are quasi-reversible[11] and symmetric ($\alpha \sim 0.5$) in nature.

Found in Figure 1, plot A, are the two-electron potentials (E_m) for **1d/2d** plotted against pH. The solid curve in this plot was computer generated from the Nernst equation,[12] employing the experimentally determined pK_a values for **1d** and **2d** found in Table I. The Nernst fit requires the hydroquinone species to have two more acid dissociations than the quinone species, and thus three quinone and five hydroquinone pK_a values are provided in the table. Assignment of the acid species corresponding to each pK_a value is based on results of our previous electrochemical studies.[2,6,8] In most cases, these pK_a values were determined spectrophotometrically in aqueous buffer, μ = 1.0 (KCl), at 30 °C. The acid dissociations shown in entries 5 and 7 are not accompanied by large absorbance chances, and the pK_a values were estimated employing the Nernst equation and Em vs pH data. The acid dissociations shown in entries 1 and 8 occur outside the pH range studied, and only approximate values are provided in Table I.

Scheme V



The Nernst fit for **1d/2d** provides, at a glance, the redox potential of the various neutral and ionic forms of this couple. Inflections in the Nernst fit reflect the pK_a values shown in Table I. The high E_0 value, 681 mV vs NHE, is due to the presence of electron-deficient protonated species: $\mathbf{1dH^+} + 3H^+ + 2e^- \rightleftarrows \mathbf{2dH_2^{2+}}$ at pH = 0. The presence of electron-rich anionic species at pH 7 result in a substantially lower potential: E_7 = 100 mV vs NHE for $\mathbf{1d^-} + 3H^+ + 2e^- \rightleftarrows \mathbf{2d}$.

The influence of the fused 2,3-dimethylimidazole ring is easily seen by comparing plot A with the Nernst fit for **13/14** shown in plot B of Figure 1. Above pH 6, the electron-releasing effect of the neutral fused 2,3-dimethylimidazole ring (pK_a of protonated form is ~4.7) decreases the potentials for **1d/2d** by 200 mV. The presence of the N(1) protonated form of this ring decreases the difference between plots A and B much below pH 6.

Quinone Methide Formation and Fate. The hydrolysis of the 6α-chloro hydroquinone derivative **2b** was studied at 5×10^{-5} M in anaerobic aqueous buffers over the pH range of 3–10 (μ = 1.0, KCl) at 30.0 ± 0.2 °C. The course of hydrolysis was followed spectrophotometrically at 372 or 420 nm, either with a stopped-flow instrument or a conventional UV–visible spectrometer. All absorbance vs time plots are first-order in character, and k_{obsd} values, obtained by computer fitting, are independent of buffer concentration. The sole product of **2b** hydrolysis at all pH values is **1d**; yields are quantitative based on final UV–visible spectra of reaction mixtures, and an 94% yield of **1d** was obtained from a preparative hydrolysis reaction (see the Experimental Section).

The mechanism put forth to explain the formation of **1d** from **2b** is outlined in Scheme V. Chloride elimination is proposed to occur by two mechanisms: spontaneous loss of chloride from **2b** to afford carbocation **16** and loss of chloride from $\mathbf{2b^-}$ to afford zwitterion **17**. The relative rates of chloride elimination from **2b** and $\mathbf{2b^-}$ species, k_2/k_1 = 28, reflects the electrostatic influence of the 9-hydroxyl anion.[13] Proton loss from **16** and a prototropic shift in **17** both afford **3**, which then ketonizes to **1d**.

(11) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; Wiley: New York, 1980; pp 227–231.

(12) For examples of multiple pK_a Nernst equations, see: Eberlein, G. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 6685.

(13) (a) Bruice, T. C.; Holmquist, B. *J. Am. Chem. Soc.* **1967**, *89*, 4082. (b) Holmquist, B.; Bruice, T. C. *J. Am. Chem. Soc.* **1969**, *91*, 2982 and 2985.

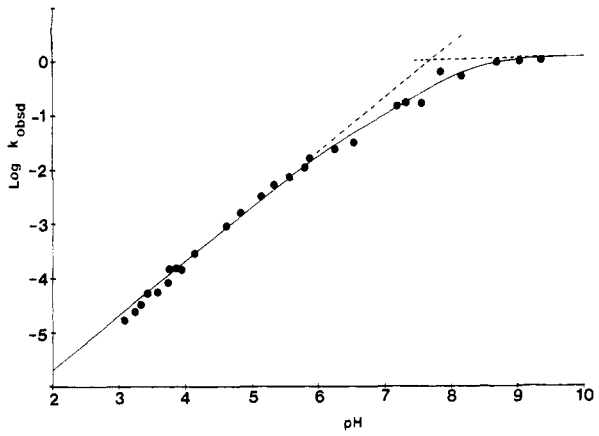


Figure 2. Plot of log k_{obsd} vs pH for the first-order hydrolysis of **2b** in anaerobic buffer (μ = 1.0 KCl) at 30.0 ± 0.2 °C.

Evidence for this mechanism is seen in the pH–rate profile for **2b** hydrolysis shown in Figure 2. At first glance, the pH–rate profile in Figure 2 appears to obey a single pK_a rate law with product formation occurring from **2b**$^-$. Data obeying this rate law would show a +1 to 0 slope change, as illustrated with dashed lines in Figure 2. Deviations of the data from the single pK_a rate law between pH 6 and 8 suggest the presence of an additional mechanism for product formation, however. The results of electrochemical studies indicate acid dissociation from the N(1)-protonated hydroquinone (**2dH**$^+$, Table I) occurs at pK_a ~ 4.7. Thus, the presence of the acid dissociation **2bH**$^+$ ⇄ **2b** + H$^+$, with product formation occurring from **2b**, is considered in the hydrolysis mechanism of Scheme V. The rate law for product formation from both **2b** and **2b**$^-$ is shown in eq 1 where k_1, k_2, K_{a_1}, and K_{a_2} are the

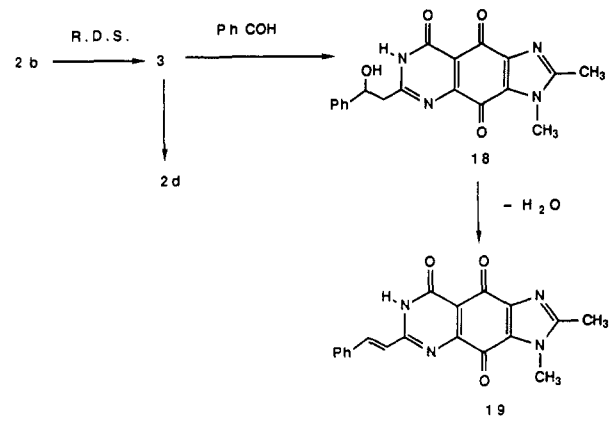
$$k_{obsd} = \frac{k_1 K_{a_1}}{a_H + K_{a_1}} + \frac{k_2 K_{a_2}}{a_H + K_{a_2}} \quad (1)$$

constants shown in Scheme V and a_H is the proton activity determined with a pH meter. Computer fitting of eq 1 to the k_{obsd} vs pH data for **2b** hydrolysis afforded the solid curve shown in Figure 2. The parameters obtained from the fit are provided in Scheme V.

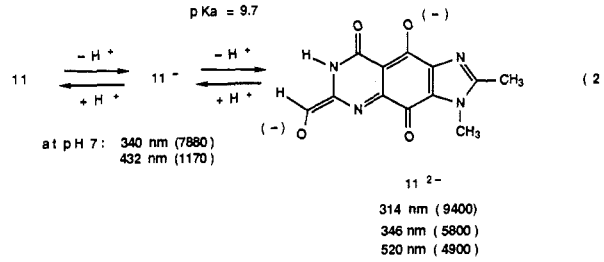
Consistent with the postulated mechanism, the kinetic pK_a value for **2b** ⇄ **2b**$^-$ + H$^+$ (pK_{a_2} = 8.2) is nearly identical with that for acid dissociation of the 9-hydroxyl proton from **2d** (8.5 in Table I). On the other hand, there is a large difference between the kinetic pK_a value for **2bH**$^+$ ⇄ **2b** + H$^+$ (pK_{a_1} = 6.5) and the value determined electrochemically for **2dH**$^+$ (pK_a = 4.7, Table I). Errors in both the electrochemical pK_a determination and the kinetic pK_a determination (due to the barely discernable plateau) probably account for the difference in values, however.

Unlike previous quinone methides studied in this laboratory,[3,6,14] **3** does not trap nucleophiles such as chloride, water, or hydroxyethyl mercaptide. The predominate reaction of **3**, even in the presence of excessive concentrations of nucleophiles, is ketonization of **1d**. This reaction pattern is similar to those of anthracycline quinone methides,[15] which actually build up in anaerobic methanol-d_1 buffered with trisma[16] and condense with benzaldehyde. To determine if **3** does likewise, a solvolysis study of **2a,b** was carried out in anaerobic buffered methanol-d_1.

Scheme VI



UV–visible spectral studies of aldehyde **11** provided insights into the probable spectral characteristics of **3** (eq 2). In strong anaerobic base, **11** is converted to the purple dianion, which likely has the methide structure **11**$^{2-}$ shown in eq 2. Evidence for anion delocalization into the aldehyde group is the absence of long wavelength λ_{max} values in strong base when the aldehyde group is replaced by methyl (i.e., **2d**$^{2-}$). Significantly, 11-deoxydaunomycin quinone methide (λ_{max} = 340 and 528 nm)[15b] has a visible spectrum similar to **11**$^{2-}$.



To provide the most optimal conditions for the buildup of **3**, the highly reactive 6α-bromo analogue **2a** was added to methanol-d_1 buffered with trisma. The deuterium solvent isotope effect should slow the ketonization reaction enough to permit buildup of **3**. Repetitive scans revealed only the formation of **1d** without the buildup of species with λ_{max} values > 450 nm, however.

The trapping of **3** with benzaldehyde in buffered methanol-d_1 was successful, yielding both the aldol product (**18**) and the dehydrated product (**19**) (Scheme VI). The rates of **2b** solvolysis in buffered methanol-d_1 are independent of benzaldehyde concentration, indicating the rate-determining formation of **3**, which is trapped by benzaldehyde in a relatively fast step.

Comparison of Quinone Methides 3 and 15. The change from an electron-deficient to an electron-rich reductive alkylating agent has two results: leaving group elimination from the hydroquinone form becomes more facile and the quinone methide is more prone to ketonization.

Chloride elimination from the quinazoline hydroquinone monoanion (**2b**$^-$ sans the fused 2,3-dimethylimidazole ring) occurs at 5.1 × 10^{-4} s^{-1} while chloride elimination from **2b**$^-$ occurs at 1.2 s^{-1}. Thus, the 200-mV decrease in reduction potential upon fusing the 2,3-dimethylimidazole ring to the quinazoline system results in a 2300-fold increase in the rate of leaving group elimination. Indeed, bromide elimination from **2a**$^-$ (should occur at 115 s^{-1} if the above

(14) Skibo, E. B. *J. Org. Chem.* **1986**, *51*, 522.
(15) (a) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 2504. (b) Kleyer, D. L.; Koch, T. H. *J. Am. Chem.* **1983**, *105*, 5154. (c) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 2387. (d) Kleyer, D. L.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 1105. (e) Boldt, M.; Gaudiano, G.; Koch, T. H. *J. Org. Chem.* **1987**, *52*, 2146.
(16) Trisma is short for tris(hydroxymethyl)aminomethane.

relationship holds) is to fast to follow. The electron-rich character of the imidazo[4,5-*g*]quinazoline hydroquinones (**2**) also results in elimination reactions not observed in the quinazoline system: hydroxide elimination from **2c**$^-$ occurs at 1×10^{-6} s^{-1}, and **2b** spontaneously eliminates chloride (process k_1 in Scheme V).

The quinone methide based on quinazoline (**15**) traps nucleophiles (water and hydroxyethyl mercaptide) irreversibly and also ketonizes to the corresponding quinone (**13**). In contrast, **3** acts only as an electrophile trap (protons and benzaldehyde) due to the low reduction potential of the imidazo[4,5-*g*]quinazoline system. Under strongly acidic conditions, this system is diprotonated and possesses a high reduction potential (see Table I and Figure 1). Consistent with our correlation of reduction potential with quinone methide reactivity, nucleophile trapping is the major product of hydrolysis under these conditions (e.g., **2a** → **2b** in Scheme II).

Conclusions

The present study shows the value of electrochemistry in predicting the behavior of a reductive alkylating agent. Reduction potential measurements are made on the quinone form of the reductive alkylating agent without the leaving group (e.g., quinone **1d** for the study of **1a,b** in Scheme I). These measurements provide insights into the facility of leaving group elimination from the reduced reductive alkylating agent, as well as the fate of the quinone methide intermediate (nucleophile trapping vs ketonization).

The rate of leaving group elimination from the reduced reductive alkylating agent increases as the reduction potential of the agent decreases. Halide leaving groups are slowly eliminated from high reduction potential agents (e.g., quinazolines, E_7 = 300 mV), whereas oxygen leaving groups are not eliminated at all. Low reduction potential agents (e.g., imidazo[4,5-*g*]quinazolines, E_7 = 100 mV) eliminate halides at stopped-flow rates and eliminate oxygen leaving groups as well. We conclude that low reduction potential alkylating agents (E_7 < 100 mV) should be substituted with weak leaving groups (acetate, carbamate, hydroxide, etc.) and that high reduction potential alkylating agents ($E_7 \gg$ 100 mV) should be substituted with excellent leaving groups (halides). In both cases, respectively, excessive reactivity and the lack of reactivity are avoided.

Lowering the reduction potential of the reductive alkylating agent favors ketonization of the quinone methide over its nucleophile trapping. Paradoxically, the best antitumor reductive alkylating agents possess very low reduction potentials,[17] which would result in ketonization of the quinone methide intermediate. Examples are the anthracycline quinone methides, which ketonize readily[15] and trap nucleophiles only under the most exacting conditions.[18] Perhaps the low reduction potential of these systems actually assists in nucleophile trapping: the unstable hydroquinone resulting from nucleophile trapping of the quinone methide can rapidly oxidize to the more stable quinone analogue and thereby prevent elimination of the nucleophile.[18] Another possibility is that low potential reductive alkylating agents covalently bind to important biological structures via electrophile trapping of the quinone methide (ketonization).

Currently, we are testing the imidazo[4,5-*g*]quinazolines **1a–c**, **4**, and analogues bearing a leaving group in the 2α-position as reductive alkylating agents in a variety of purine-utilizing enzyme systems. The results of these studies will be reported in due course.

Experimental Section

All analytically pure compounds were dried under high vacuum in a drying pistol heated with refluxing methanol. Some of these compounds still contained water of crystallization that was determined from the elemental analyses found. Uncorrected melting and decomposition points were determined with a Mel-Temp apparatus. All TLC's were run with Merck silica gel 60 (F_{254}) plates employing a variety of solvents. ^{1}H NMR spectra were obtained at 90 MHz.

pK_a constants were determined by spectrophotometric titration in μ = 1.0 (KCl) aqueous solvent at 30 ± 0.2 °C employing a Perkin-Elmer 559 or Lambda-3 spectrometer. Measurements were usually carried out under aerobic conditions; 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.[19]

Kinetic Studies of Hydrolysis. The hydrolytic studies of **2b** were carried out in aqueous buffer at 30.0 ± 0.2 °C under an argon atmosphere with Thunberg cuvettes. A dimethyl sulfoxide stock of **2b** was placed in the top port, and the aqueous buffer was placed in the bottom port. After a stream of purified argon was passed into each port for 30 min, the cuvette was sealed and equilibrated at 30 °C in a thermostated cell holder for 20 min. The ports were then mixed and absorbance vs time data obtained with a Perkin-Elmer 559 or Lambda-3 UV–vis spectrometer. Reactions proceeding at k_{obsd} > 0.2 s^{-1} were followed with a Durham stopped-flow instrument set up in a nitrogen glovebox. Absorbance vs time data were computer fit to a first-order rate law.

Electrochemistry. Determination of E_m values was carried out with a BAS 27 voltamograph. The working electrode material was glassy carbon and the reference electrode was Ag/AgCl, which was calibrated against benzoquinone, E_0 = 699 mV (NHE).[20] Measurements were carried out in μ = 1.0 ($NaClO_4$) aqueous buffer at 25–26 °C under an atmosphere of argon employing scan speeds of 100 mV s^{-1}. 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 the redox couple **1d/2d**, 26 E_m determinations were made over the pH range studied. For each E_m value, an E_0 value was calculated from the Nernst equation, containing the acid dissociation constants in Table I and the proton activity determined with a pH meter. The average of all E_0 determinations was substituted into the Nernst equation, with which the solid curve in Figure 1 was generated.

Synthesis and physical properties of new compounds are provided below.

Ethyl 6-amino-1-methyl-2-(methoxymethyl)benzimidazole-5-carboxylate (5b) was prepared from ethyl 3-amino-4-(methylamino)benzoate[21] by the three-step reaction sequence described below.

To a solution of 5.1 g (22 mmol) of ethyl 3-amino-4-(methylamino)benzoate in 50 mL of dry benzene, containing 0.5 mL of dry pyridine, was added 3.5 g (42 mmol) of methoxyacetyl chloride over a period of 10 min. The reaction mixture was then stirred for 15 h at room temperature. The blue-white solid that formed was collected and washed with hexane. Recrystallization was carried out by dissolving the solid in 50 mL of hot ethanol, filtering and discarding the solids, and then combining the filtrate with 50 mL of hexane. Cooling the mixture in a refrigerator overnight afforded pure ethyl 1-methyl-2-(methoxymethyl)benzimidazole-5-carboxylate as the HCl salt: yield 4.0 g (73%); TLC (acetone) R_f = 0.5; mp 178 °C; IR (KBr) 1736, 1717, 1526, 1311, 1279, 1199, 1125, 1075, 761, 742 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 7.92–8.17 (2 H,

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multiplet, 6-H and 7-H), 8.30 (1 H, multiplet, 4-H), 4.98 (2 H, s, 2-methylene), 4.37 (2 H, q, J = 7 Hz, methylene of ester), 3.95 (3 H, s, N(1)-methyl), 3.47 (3 H, s, 2α-methoxy), 1.37 (3 H, t, J = 7 Hz, methyl of ester); mass spectrum (EI mode) m/z 248 (P^+). Anal. Calcd for $C_{13}H_{16}N_2O_3$·HCl: C, 54.83; H, 6.02; N, 9.84. Found: C, 54.84; H, 6.05; N, 9.98.

The compound obtained above was nitrated by adding 5.0 g (50.6 mmol) portionwise to 20 mL of an ice-cold mixture of concentrated sulfuric acid/90% nitric acid (50:50) over a period of 15 min. The reaction mixture was stirred for 1 h at room temperature and then poured unto 250 g of ice. The resulting solution was neutralized with NH_4OH and then extracted with 3 × 50 mL of chloroform. The dried chloroform extracts ($MgSO_4$) were evaporated to a small volume, from which analytically pure ethyl 1-methyl-2-(methoxymethyl)-6-nitrobenzimidazole-5-carboxylate was obtained by addition of hexane and chilling: yield 3.9 g (64%); mp 95–96 °C; TLC (acetone) R_f = 0.62; IR (KBr) 1723, 1529, 1369, 1342, 1336, 1281, 1263, 1095, 1027, 846 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.47 (1 H, s, 7-H), 8.04 (1 H, s, 4-H), 4.78 (2 H, s, 2-methylene), 4.31 (2 H, q, J = 7.1 Hz, methylene of ester), 3.93 (3 H, s, N(1)-methyl), 3.36 (3 H, s, 2α-methoxy), 1.29 (3 H, t, J = 7.1 Hz, methyl of ester); mass spectrum (EI mode) m/z 293 (P^+). Anal. Calcd for $C_{13}H_{15}N_3O_5$: C, 53.24; H, 5.15; N, 14.32. Found: C, 53.38; H, 5.13; N, 14.30.

A solution of the nitro derivative obtained above (2.4 g, 8 mmol) in methanol (100 mL), containing 0.1 g of 5% Pd on carbon, was shaken under 45 psi of H_2 for 3.5 h. The reaction mixture was then filtered through Celite, and the filtrate was concentrated to a solid in vacuo. Recrystallization was carried out by dissolving **5b** in 5 mL of absolute ethanol and then diluting this solution with 5 mL of hexane: yield 1.4 g (66%); mp 125 °C; TLC (acetone) R_f = 0.25; IR (KBr) 3429, 1685, 1639, 1445, 1361, 1295, 1244, 1189, 1178, 1101 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 8.02 (1 H, s, 4-H), 6.67 (1 H, s, 7-H), 6.47 (2 H, s, Amino), 4.60 (2 H, s, 2-methylene), 4.28 (2 H, q, J = 7.1 Hz, methylene of ester), 3.63 (3 H, s, N(1)-methyl), 3.31 (3 H, s, 2α-methoxy), 1.33 (3 H, t, J = 7.1 Hz methyl of ester); mass spectrum (EI mode) m/z 263 (P^+). Anal. Calcd for $C_{13}H_{17}N_3O_3$: C, 59.30; H, 6.51; N, 15.95. Found: C, 59.09; H, 6.43; N, 16.03.

Ethyl 6-(α-Methoxyacetamido)-1,2-dimethylbenzimidazole-5-carboxylate (6a). To a solution of 2.3 g (9.86 mmol) of **5a**[21] in 50 mL of benzene was added 1.2 mL of pyridine and 1.2 mL (12.5 mmol) of methoxyacetyl chloride. The reaction mixture was stirred for 12 h, and then the benzene was removed in vacuo to afford a solid residue. Addition of ~50 mL of water to this residue and adjustment of the pH to 8 with Na_2CO_3, while chilling in an ice bath, afforded crude **6a**. Recrystallization was carried out from aqeuous ethanol: yield 2.4 g (81%); mp 174–175 °C; TLC [1-butanol, acetic acid, water (5:2:3)] R_f = 0.53; ^1H NMR (Me_2SO-d_6) δ 8.70 and 8.20 (2 H, 2 s, 7-H and 4-H, no assignments made), 4.36 (2 H, q, J = 8 Hz, methylene of ester), 4.06 (2 H, s, methylene of acetamido), 3.70 (3 H, s, N(2)-methyl), 3.46 (3 H, s, methoxy), 2.54 (3 H, s, 2-methyl), 1.37 (3 H, t, J = 8 Hz, methyl of ester); IR (KBr) 3348, 1696, 1682, 1600, 1522, 1288, 1231, 1221, 1197, 1181, 1122 cm^{-1}; mass spectrum (EI mode) m/z 305 (P^+). Anal. Calcd for $C_{15}H_{19}N_3O_4$·0.25H_2O: C, 58.14; H, 6.34; N, 13.55. Found: C, 58.37; H, 6.24; N, 13.61.

Ethyl-6-(α-Methoxyacetamido)-2-(methoxymethyl)-1-methylbenzimidazole-5-carboxylate (6b). To a mixture of **5b** (1.3 g, 4.99 mmol) in 20 mL of dry benzene was added 0.5 mL of pyridine and 0.7 g (6.4 mmol) of methoxyacetyl chloride. The reaction mixture was stirred for 3 h at room temperature and then combined with 100 mL of water. The aqueous layer was made slightly basic with saturated aqueous sodium bicarbonate, and the benzene layer was separated. The aqueous layer was then extracted with 3 × 50-mL portions of chloroform. The combined organic layers were dried ($MgSO_4$) and then evaporated to a ~10-mL volume. Dilution of this volume with 50 mL of hexane afforded **6b** (1.36 g, 83%) as a fibrous, white solid. Analysis and characterization were carried out on **6b**·HCl: mp 177–178 °C; TLC (acetone) R_f = 0.4; ^1H NMR (Me_2SO-d_6) δ 8.86 and 8.33 (2 H, 2 s, 4-H and 7-H, no assignments made), 4.84 (2 H, s, 2-methylene), 4.31 (2 H, q, J = 7.1 Hz, methylene of ester), 4.08 (2 H, s, methylene of 6-(methoxyacetamido)), 3.82 (3 H, s, N(1)-methyl), 3.47 (3 H, s, methoxy of 6-(methoxyacetamido)), 3.40 (3 H, s, 2α-methoxy), 1.38 (3 H, t, J = 7.1 Hz, methyl of ester); IR (KBr) 3250, 1702, 1683, 1585, 1516, 1470, 1286, 1229, 1117, 1062 cm^{-1}; mass spectrum (EI mode), m/z 335 (P^+ of base). Anal. Calcd for $C_{16}H_{21}N_3O_5$·HCl·H_2O: C, 49.29; H, 5.94; N, 10.77. Found: C, 49.65; H, 6.11; N, 10.98.

Ethyl 6-(α-Methoxyacetamido)-1,2-dimethyl-7-nitrobenzimidazole-5-carboxylate (7a). To 25 mL of an ice-cold mixture of 90% HNO_3/H_2SO_4/acetic acid (3:1:1) was added 1.80 g (5.89 mmol) of **6a** portionwise. The reaction mixture was then allowed to stir at room temperature for 2.5 h. Workup consisted of pouring the reaction mixture into 50 mL of cold water, neutralization with concentrated NH_4OH, extraction of the neutralized solution with 3 × 100 mL of chloroform, and evaporation of the dried extracts (Na_2SO_4) to afford crude **7a**. Recrystallization was carried out by dissolving **7a** in ethanol and then diluting this solution with a small volume of hexane: yield 1.5 g (73%); mp 119–121 °C; TLC [1-butanol, acetic acid, water (5:2:3)] R_f = 0.63; ^1H NMR (Me_2SO-d_6) δ 8.25 (1 H, s, 7-H), 4.29 (2 H, q, J = 8 Hz, methylene of ester), 3.98 (2 H, s, methylene of 6-(α-methoxyacetamido)), 3.58 (3 H, s, N(1)-methyl), 3.40 (3 H, s, methoxy), 2.61 (3 H, s, 2-methyl), 1.32 (3 H, t, J = 8 Hz, methyl of ester); IR (KBr) 3289, 1701, 1533, 1500, 1433, 1367, 1284, 1231, 1227, 1208, 1109 cm^{-1}; mass spectrum (EI mode), m/z 350 (P^+). Anal. Calcd for $C_{15}H_{18}N_4O_6$: C, 51.43; H, 5.18; N, 15.99. Found: C, 51.39; H, 5.15; N, 16.02.

Ethyl 6-(α-Methoxyacetamido)-2-(methoxymethyl)-1-methyl-7-nitrobenzimidazole-5-carboxylate (7b). To 8 mL of an ice-cold solution of 90% nitric acid/sulfuric acid (1:1) was added 2 g (5.96 mmol) of **6b** over a period of 5 min. The reaction mixture was stirred at room temperature for 1 h and poured over 100 g of ice. The solution was made neutral with satruated aqueous sodium bicarbonate and extracted four times with 50-mL portions of chloroform. The extracts were dried over Na_2SO_4 and then evaporated to 10 mL in vacuo. Addition of hexane and chilling afforded **7b** as a crystalline yellow solid: yield 1.85 g (81%); mp 89–90 °F; TLC (acetone) R_f = 0.63; ^1H NMR (Me_2SO-d_6) δ 8.37 (1 H, s, 4-H), 4.78 (2 H, s, 2-methylene), 4.29 (2 H, q, J = 7.1 Hz, methylene of ester), 3.99 (2 H, s, methylene of 6-(methoxyacetamido)), 3.35 and 3.40 (6 H, 2 s, methoxy groups), 3.66 (3 H, s, N(1)-methyl), 1.32 (3 H, t, J = 7.1 Hz methyl of ester); IR (KBr) 3280, 1710, 1695, 1541, 1531, 1502, 1442, 1367, 1249, 1111, 1083 cm^{-1}; mass spectrum (EI mode), m/z 380 (P^+). Anal. Calcd for $C_{16}H_{20}N_4O_7$: C, 50.52; H, 5.30; N, 14.72. Found: C, 50.40; H, 5.24; N, 14.64.

2,3-Dimethyl-6-(methoxymethyl)-4-nitroimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (8a) and 3-Methyl-2,6-bis(methoxymethyl)-4-nitroimidazo[4,5-*g*]quinazolin-8(3*H*,7H)-one (8b). A solution of **7a,b** (3 mmol) in 50 mL of methanol was chilled in a dry ice/acetone bath and then combined with 15 mL of liquid ammonia. The reaction mixture was then removed from the cooling bath and stirred at room temperature for 8 h. The ammonia addition and stirring sequence was repeated two more times, and the reaction was worked up.

For **8a**, the reaction mixture was evaporated to dryness and the solid was recrystallized from hot ethanol: 86% yield; mp >298 °C dec; TLC [1-butanol, acetic acid, water (5:2:3)] R_f = 0.61; ^1H NMR (Me_2SO-d_6) δ 8.38 (1 H, s, 9-H), 4.34 (2 H, s, methylene), 3.64 (3 H, s, N(3)-methyl), 3.38 (3 H, s, methoxy), 2.64 (3 H, s, 2-methyl); IR (KBr) 3172, 3080, 1661, 1623, 1534, 1470, 1421, 1374, 1125, 771 cm^{-1}; mass spectrum (EI mode), m/z 303 (P^+). Anal. Calcd for $C_{13}H_{13}N_5O_4$: C, 51.49; H, 4.32; N, 23.09. Found: C, 51.23; H, 4.27; N, 22.89.

Crystallization of **8b** occurred from the reaction mixture in analytically pure form: 81% yield; mp >274 °C dec; TLC (acetone) R_f = 0.51; ^1H NMR (Me_2SO-d_6) δ 8.52 (1 H, s, 9-H), 4.80 (2 H, s, 2-methylene), 4.35 (2 H, s, 6-methylene), 3.72 (3 H, s, N(3)-methyl), 3.38 (3 H, s, 6α-methoxy), 3.29 (3 H, s, 2α-methoxy); IR (KBr) 1690, 1633, 1615, 1526, 1411, 1396, 1354, 1123, 1089, 794 cm^{-1}; mass spectrum (EI mode), m/z 333 (P^+). Anal. Calcd for $C_{14}H_{15}N_5O_5$: C, 50.44; H, 4.53; N, 21.01. Found: C, 50.66; H, 4.49; N, 20.93.

4-Amino-6-(methoxymethyl)-2,3-dimethylimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (9a). To a refluxing solution of **8a** (500 mg, 1.65 mmol) in 100 mL of methanol was added a solution of $Na_2S_2O_4$ (250 mg) in 1 mL of water. Addition of this portion of $Na_2S_2O_4$ was repeated until TLC revealed only the presence of **9a**. The reaction mixture was then cooled to room temperature,

and the solids were filtered off. Evaporation of the filtrate to a solid residue and addition of ~5 mL of cold water afforded pure **9a** as a white solid: yield 320 mg (71%); mp 267–269 °C dec; TLC [1-butanol, acetic acid, H_2O (5:2:3)] R_f = 0.39; ^1H NMR (Me_2SO-d_6) δ 7.57 (1 H, s, 9-H), 4.37 (2 H, s, methylene), 4.11 (3 H, s, N(3)-methyl), 3.39 (3 H, s, methoxy), 2.67 (3 H, s, 2-methyl); IR (KBr) 3457, 3360, 2829, 1706, 1684, 1640, 1617, 1591, 1467, 1434 cm^{-1}; mass spectrum (EI mode), m/z 273 (P^+). Anal. Calcd for $C_{13}H_{15}N_5O_2 \cdot 0.25H_2O$: C, 56.21; H, 5.62; N, 25.20. Found: C, 55.97; H, 5.18; N, 25.32.

4-Amino-2,6-bis(methoxymethyl)-3-methylimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (9b). A solution of **8b** (1 g, 3 mmol) in 250 mL of methanol, containing 200 mg of Pd on carbon, was shaken under 45 psi of H_2 for 2 h. The catalyst was then removed by filtration through Celite, and the crystallized **9b** was washed into the filtrate with aqueous acetic acid. Concentration of the filtrate to remove methanol solvent and neutralization with satruated aqueous sodium bicarbonate afforded pure **9b** as a white crystalline solid: yield 700 mg (77%); mp >260 °C dec; TLC (1-butanol, acetic acid, water [5:2:3]) R_f = 0.4; ^1H NMR (Me_2SO-d_6) δ 7.64 (1 H, s, 9-H), 4.69 (2 H, s, 2-methylene), 4.34 (2 H, s, 6-methylene), 4.12 (3 H, s, N(3)-methyl), 3.34 and 3.38 (6 H, 2 s, methoxys); IR (KBr) 3309, 2936, 1679, 1663, 1636, 1610, 1467, 1100, 1093 cm^{-1}; mass spectrum (EI mode), m/z 303 (P^+). Anal. Calcd for $C_{14}H_{17}N_5O_3$: C, 55.22; H, 5.69; N, 23.09. Found: C, 55.43; H, 5.71; N, 22.88.

6-(Bromomethyl)-2,3-dimethylimidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (1a). A solution of **9a** (83 mg, 0.3 mmol) in 5 mL of 48% HBr was heated to 120 °C. At 1-h intervals with continued heating, dry HBr gas was introduced into the reaction mixture for a period of 10 min; a total of six HBr additions were made. The hot reaction mixture was poured into 20 mL of ethyl acetate and chilled for 1 h. The resulting golden-yellow solid was filtered and dried to yield 111 mg of a 70:30 mixture (by ^1H NMR) of the 6α-bromo and 6α-hydroxy derivatives, respectively: ^1H NMR (Me_2SO-d_6) δ 7.56 and 7.53 (9-H of 6α-hydroxy and 6α-bromo, respectively), 4.45 and 4.48 (methylene of 6α-hydroxy and 6α-bromo, respectively), 4.18 (N(3)-methyl of both derivatives), 2.81 (2-methyl of both derivatives).

To a suspension of the crude bromomethyl amine (92 mg) in 20 mL of water, containing 150 mg of monobasic potassium phosphate, was added a solution of potassium nitrosodisulfonate (430 mg) and monobasic potassium phosphate (400 mg) in 30 mL of water. The resulting reaction mixture was then stirred for 1.5 h at room temperature. The completed reaction was extracted with chloroform (3 × 30 mL), and the extracts were dried (Na_2SO_4) and evaporated to an oily residue. Addition of ~5 mL of methanol to the residue and chilling for 1 h resulted in crystallization of orange-red **1a**: yield 13.9 mg (26% based on **9a**); mp >155 °C dec; TLC [1-butanol, acetic acid, H_2O (5:2:3)] R_f = 0.44; ^1H NMR (Me_2SO-d_6) δ 4.43 (2 H, s, bromomethyl), 3.88 (3 H, s, N(3)-methyl), 2.47 (3 H, s, 2-methyl); IR (KBr) 3419, 3236, 3218, 3211, 2919, 1705, 1682, 1578, 1524, 1473 cm^{-1}; mass spectrum (EI, solids probe), m/z 336 (P^+, ^{79}Br), 338 (P^+, ^{81}Br). Anal. Calcd for $C_{12}H_9BrN_4O_3 \cdot H_2O$: C, 40.58; H, 3.12; N, 15.78. Found: C, 40.67; H, 2.66; N, 15.60.

2,6-Bis(bromomethyl)-3-methylimidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (4). The procedure described for the preparation of **1a** from **9a** was employed for the preparation of **4** from **9b**.

The crude yield of the 2,6-bis(bromomethyl) analogue of **9b**, as its HBr salt, was 81%: ^1H NMR (Me_2SO-d_6) δ 7.65 (1 H, s, 9-H), 5.11 and 4.47 (4 H, 2 s, 2- and 6-bromomethyls, respectively), 4.24 (3 H, s, N(3)-methyl).

The 2,6-bis(bromomethyl) derivative obtained above was oxidized to **4** in 27% yield (based on **9b**): mp >300 °C dec; TLC (1-butanol, acetic acid, water [5:2:3]) R_f = 0.46; ^1H NMR (Me_2SO-d_6) δ 4.92 and 4.44 (4 H, 2 s, 2- and 6-bromomethyls, respectively), 3.97 (3 H, s, N(3)-methyl); IR (thin film on KBr) 1714, 1684, 1570, 1533, 1465 cm^{-1}; mass spectrum (EI mode, solids probe), m/z 414, 416, 418 (P^+ for the ^{79}Br, ^{79}Br; ^{81}Br, ^{79}Br; and ^{81}Br, ^{81}Br combinations, respectively, at an intensity ratio of 1:2:1). Anal. Calcd for $C_{12}H_9Br_2N_4O_3 \cdot 0.25H_2O$: C, 34.26; H, 2.03; N, 13.31. Found: C, 34.24; H, 2.41; N, 12.91.

6-(Bromomethyl)-4,9-dihydroxy-2,3-dimethylimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (2a). A mixture of **1a** (5.3 mg, 0.016 mmol) in 0.2 mL of 33% HBr in acetic acid was stirred for 3 h at room temperature. The yellow **2a**·2HBr crystallized from the reaction mixture: yield 5.0 mg (63%) upon filtration and drying; mp >260 °C dec; ^1H NMR (Me_2SO-d_6) δ 4.44 (2 H, s, bromomethyl), 4.13 (3 H, s, N(3)-methyl), 2.77 (3 H, s, 2-methyl); IR (KBr) 3397, 2630, 1682, 1617, 1510, 1467, 1366, 1242, 1151 cm^{-1}. Anal. Calcd for $C_{12}H_{11}BrN_4O_3 \cdot 2HBr$: C, 28.75; H, 2.61; N, 11.17. Found: C, 28.31; H, 2.80; N, 10.82.

6-(Chloromethyl)-4,9-dihydroxy-2,3-dimethylimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (2b). To a solution consisting of 0.05 mL of Me_2SO and 0.2 mL of concentrated HCl was added 7.9 mg (0.016 mmol) of **2a**·2HBr. The reaction mixture was stirred for 2 h at room temperature and the crystallized **2b**·2HBr salt filtered off: yield after washing with acetone and drying was 4.0 mg (56%): IR (KBr) 3500, broad band 2700–2500, 1685, 1669, 1619, 1471, 1160, 1150 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 4.59 (2 H, s, chloromethyl), 4.13 (3 H, s, N(3)-methyl), 2.77 (3 H, s, 2-methyl); mass spectrum (EI mode), m/z 286 (P^+), 80 and 82 (HBr isotopes). Anal. Calcd for $C_{12}H_{11}ClN_4O_3 \cdot 2HBr$: C, 31.57; H, 2.87; N, 12.27. Found: C, 31.71; H, 2.91; N, 12.23.

2,3-Dimethyl-6-(methoxymethyl)imidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (10). To a solution consisting of 90.4 mg (0.33 mmol) of **9a** in 60 mL of 50% aqueous acetone was added a solution consisting of 800 mg of KH_2PO_4 and 700 mg of Fremy's salt in 50 mL of water. The reaction mixture was stirred overnight and then concentrated in vacuo to ~20 mL. After adusting the pH to 6 with KOH, the quinone **10** was extracted with 3 × 50 mL of chloroform. The chloroform extracts were dried (Na_2SO_4) and then evaporated to ~5 mL. Addition of hexane afforded **10** as a yellow-green solid: yield 69 mg (72%); mp >220 °C dec; TLC [1-butanol, acetic acid, water (5:2:3)] R_f = 0.26; ^1H NMR (Me_2SO-d_6) δ 4.18 (2 H, s, methylene), 3.85 (3 H, s, N(3)-methyl), 3.33 (3 H, s, methoxy), 2.41 (3 H, s, 2-methyl); IR (KBr) 3055, 2956, 1703, 1635, 1577, 1557, 1535, 1519, 1461, 1132 cm^{-1}; mass spectrum (EI mode), m/z 290 (P^+ + 2). Anal. Calcd for $C_{13}H_{12}N_4O_4 \cdot 0.5H_2O$: C, 52.53; H, 4.41; N, 18.84. Found: C, 52.33; H, 4.15; N, 18.35.

4,9-Dihydroxy-6-(methoxymethyl)-2,3-dimethylimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (12). A solution of 78 mg (0.271 mmol) of **10** in 3 mL of 33% HBr in acetic acid was stirred for 12 h at room temperature. The crystallized **12**·2HBr was filtered off and washed with acetone: yield 78 mg (63%); mp >300 °C dec; ^1H NMR (Me_2SO-d_6) δ 4.40 (2 H, s, methylene), 4.13 (3 H, s, N(3)-methyl), 3.39 (3 H, s, methoxy), 2.77 (3 H, s, 2-methyl); IR (KBr) 2915, 2871, 1673, 1559, 1501, 1456, 1361, 1236, 1204, 1177, 1137 cm^{-1}; mass spectrum (EI mode), m/z 290 (P^+). Anal. Calcd for $C_{13}H_{14}N_4O_4 \cdot 2HBr \cdot 0.25H_2O$: C, 34.20; H, 3.64; N, 12.27. Found: C, 33.99; H, 3.35; N, 11.56.

6-Formyl-4,9-dihydroxy-2,3-dimethylimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (11). A solution of 20 mg (0.069 mmol) of **10** was added to 2 mL of 48% HBr and heated to 110 °C for 3 h. The reaction mixture was then poured into 75 mL of ethyl acetate, and the resulting mixture was chilled for several hours. The yellow crystalline **11**·2HBr was filtered, washed with acetone, and dried: yield 21 mg (70%); mp >278 °C dec; ^1H NMR (Me_2SO-d_6) δ 9.61 (1 H, s, formyl), 4.15 (3 H, s, N(3)-methyl), 2.79 (3 H, s, 2-methyl); ^1H NMR (Me_2SO-d_6 + D_2O) δ 5.61 (1 H, s, acetal), 4.14 (3 H, s, N(3)-methyl), 2.78 (3 H, s, 2-methyl); IR (KBr) 3382, 3179, 3045, 1674, 1636, 1606, 1441, 1363, 1236, 1048 cm^{-1}; mass spectrum (EI mode), m/z 274 (P^+). Anal. Calcd for $C_{12}H_{10}N_4O_4 \cdot 2HBr$: C, 33.05; H, 2.77; N, 12.85. Found: C, 33.04; H, 3.27; N, 12.59.

4,9-Dihydroxy-6-(hydroxymethyl)-2,3-dimethylimidazo[4,5-*g*]quinazolin-8(3*H*,7*H*)-one (2c). A solution of 18.4 mg (0.04 mmol) of **12** in 1 mL of 48% HBr was heated at 120 °C for 6 h. The reaction mixture was then poured into 10 mL of ethyl acetate and chilled overnight. The yellow product was filtered off and dried: yield 14.5 mg (78%); mp >280 °C dec; ^1H NMR (Me_2SO-d_6) δ 4.47 (2 H, s, hydroxymethyl), 4.14 (3 H, s, N(3)-methyl), 2.77 (3 H, s, 2-methyl); IR (KBr) 3029, 2908, 2867, 1700, 1671, 1616, 1565, 1371, 1166 cm^{-1}; mass spectrum (EI mode), m/z 276 (P^+). Anal. Calcd for $C_{12}H_{12}N_4O_4 \cdot 2HBr \cdot 1.5H_2O$: C, 30.99; H, 3.68; N, 12.05. Found: C, 30.63; H, 3.31; N, 11.77.

Isolation of the 2a,b Hydrolysis Product, 2,3,6-Trimethylimidazo[4,5-*g*]quinazoline-4,8,9(3*H*,7*H*)-trione (1d), and Preparation of the Hydroquinone (2d). To 50 mL of pH

8 potassium phosphate buffer was added 90 mg (0.18 mmol) of **2a** (or **2b**) under strict anaerobic conditions. The reaction mixture was then stirred for 10 min at room temperature. After this time, the reaction was opened to the air and made basic (pH ~10) with KOH. The brick-red potassium salt of **1d** crystallized from solution upon chilling, yield 50 mg (94%). The salt was dissolved in 2 mL of water containing 2 drops of concentrated HCl, chilling of this solution afforded **1d** as a yellow crystalline solid: mp >300 °C dec; TLC (1-butanol, acetic acid, water [5:2:3]) R_f = 0.29; ^{1}H NMR (Me_2SO-d_6) δ 3.87 (3 H, s, N(3)-methyl), 2.46 and 2.42 (6 H, 2 s, 2- and 6-methyls, no assignments made); IR (KBr) 3450, 3280, 2946, 2923, 1700, 1566, 1470, 1125, 1041 cm^{-1}.

Conversion of **1d** to the hydroquinone dihydrobromide **2d**·2HBr was carried out using the procedure for the preparation of **12**. Physical properties of **2d**·2HBr are as follows: mp 299–301 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.12 (3 H, s, N(3)-methyl), 2.77 and 2.45 (6 H, 2 s, 2- and 6-methyls, no assignment made); mass spectrum (EI mode), m/z 260 (P^+). Anal. Calcd for $C_{12}H_{12}N_4O_3$·2HBr·0.5H_2O: C, 33.43; H, 3.51; N, 12.99. Found: C, 33.33; H, 3.41; N, 12.00.

Benzaldehyde Trappings Products of 3. To 60 mL of methanol-d_1, buffered with 4.3 × 10^{-2} M trisma (50:50, base and HCl salt) and containing 4.5 mL of benzaldehyde, was added 37 mg (0.074 mmol) of **2a** (or **2b**) under strict anaerobic conditions. The mixture was stirred at room temperature for 1 h and then opened to the air. Evaporation in vacuo to a solid residue was followed by extraction of the residue with 2 × 5 mL of chloroform. The chloroform extracts were evaporated to a small volume (~1 mL) and diluted with hexane, which resulted in precipitation of an orange solid. Mass spectral studies (EI mode) and ion intensity plots showed the solid to be **19** (m/z 348 (P^+ + 2)), with a trace amount of **18** (362 (P^+ + 2)). The P^+ + 2 peaks are the result of quinone to hydroquinone reduction by the solvent used to introduce the sample on the probe.

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Convergent and Efficient Palladium-Effected Synthesis of 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid (DDATHF)

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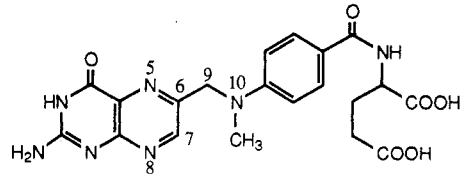
A new nine-step synthesis of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid, an extraordinarily active and selective antitumor agent now in preclinical trial, is described which utilizes two successive palladium-catalyzed carbon–carbon coupling reactions to form the C_6–C_7 and C_{10}–aryl bonds.

Methotrexate (1) has a long and distinguished history as an antineoplastic and immunosuppressive drug, but its extreme toxicity severely limits its clinical effectiveness. Methotrexate is an inhibitor of dihydrofolate reductase, which plays a critical role in many different phases of mammalian metabolism. Attempts to discern significant differences between dihydrofolate reductases derived from tumors, bacteria, and normal mammalian cells have not been rewarding, with the consequence that methotrexate, as well as all other dihydrofolate reductase inhibitors currently used clinically, are nonselective in their cytotoxicity.[1] Several years ago we reported the synthesis and preliminary biological evaluation of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, **2**), the lead compound of a new class of folate antagonists designed to be inhibitors of folate metabolism at sites other than dihydrofolate reductase.[2-4] For reasons which have been fully documented elsewhere,[5] our target was glycinamide ribonucleotide transformylase, which mediates the first formyl transfer step in the de novo purine biosynthetic pathway and which utilizes 10-formyl-5,6,7,8-tetrahydrofolic acid as its cofactor. In the event, DDATHF has been shown to possess extraordinary and selective antitumor activity; indeed, both its therapeutic index and its broad spectrum of activity against solid tumors are unrivaled among known antitumor agents.[3-10] Since DDATHF is not a DHFR inhibitor, it is fully active against tumors which have developed resistance to DHFR inhibitors such as methotrexate. DDATHF is now in preclinical trial.



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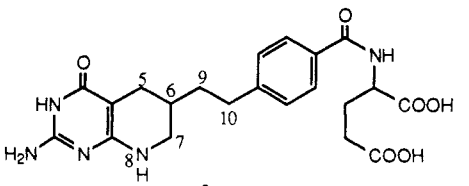

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