^{1}H NMR δ (CHCN) 6.36 [(1*R*,3-*cis*,1′*R*)-**5b** (minor)] and 6.38 [(1*R*,3-*cis*,1′*S*)-**5b** (major)].

Acknowledgment. We thank Sumitomo Chemical Co., Ltd. (Osaka, Japan), for the generous gift of the aldehydes **1f,g**, (*S*)-2-(4-chlorophenyl)-3-methylbutanoic acid, and (1*R*,*trans*/*cis*)-chrysanthemic acid, as well as for HPLC analyses of the pyrethroids **5a** and **5b**. We appreciate the generous gift of the lipase from Amano Pharm. Co., Ltd. (Nagoya, Japan). We thank Mr. Akihiko Hatanaka (Shinshu University) and Mr. Mitsuo Mimura (Kaken Pharmaceutical Co., Ltd.) for their assistance in the experiment.

Supplementary Material Available: Physical and spectral data for **3b-n′**, fenvalerate (**4**), and the chrysanthemates **5a,b** and a plot of the time course of the racemization of optically active cyanohydrin **2e** (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Design of Pyrimido[4,5-*g*]quinazoline-Based Anthraquinone Mimics. Structure–Activity Relationship for Quinone Methide Formation and the Influence of Internal Hydrogen Bonds on Quinone Methide Fate

Robert H. Lemus and Edward B. Skibo*,[1]

Department of Chemistry, Arizona State University, Tempe, Arizona 85287-1604

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Pyrimido[4,5-*g*]quinazolinequinone derivatives were synthesized as anthraquinone-like reductive alkylating agents. Like many naturally-occurring antibiotics, these quinone derivatives are designed to afford an alkylating quinone methide species upon reduction and leaving-group elimination. Kinetic studies of pyrimido[4,5-*g*]-quinazoline hydroquinones provided evidence of quinone methide intermediates able to trap nucleophiles (alkylation) and protons (ketonization). The rate of quinone methide formation is determined by the hydroquinone free energy. Thus, a linear free energy relationship for quinone methide formation was obtained by plotting rates of quinone methide formation as the log versus the quinone reduction potential. The pyrimido[4,5-*g*]quinazoline quinone methides fall on this free energy plot, showing that these species are formed by the same mechanism as the other structurally-diverse quinone methides previously studied in this research group. Internal hydrogen bonds present in pyrimido[4,5-*g*]quinazoline derivatives influence the fate of the quinone methide species as well as the rate of hydroquinone oxidation in the presence of oxygen. Such hydrogen bonds stabilize the hydroquinone species, thereby resulting in slow rates of hydroquinone oxidation to quinone in alkaline aerobic buffer. Stabilization of the hydroquinone also results in substantial nucleophile trapping by the quinone methide. Without internal hydrogen bonds, hydroquinone oxidations are rapid and the quinone methide traps only electrophiles.

Efforts in this laboratory have been directed toward the design and study of reductive alkylating agents based on heterocyclic ring systems.[2-7] Reductive alkylating agents are quinones functionalized with a leaving group so as to permit quinone methide formation upon quinone reduction. The quinone methide species can trap nucleophiles (alkylation) as well as electrophiles (ketonization). The low reduction potentials exhibited by some tumor cells[8] have generated an interest in reductive alkylating agents as selective antitumor drugs.[9] Indeed, many naturally occurring antitumor drugs may act as reductive alkylating agents.[10]

Chart I

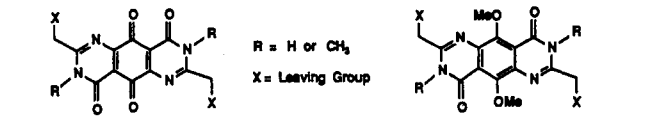
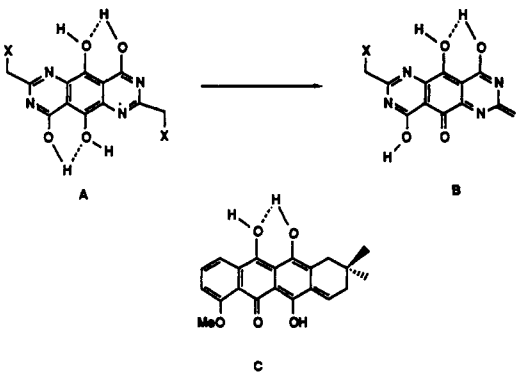


Chart II



The subjects of this paper are the synthesis, physical chemistry, and cytotoxic properties of the pyrimido[4,5-*g*]quinazoline alkylating agents in Chart I. The pyrimido[4,5-*g*]quinazolinetetrone derivatives were designed as reductive alkylating agents while the dione derivatives were

(1) National Institutes of Health research career development award recipient (CA01349), 1988–1993.
(2) Lee, C.-H.; Gilchrist, J. H.; Skibo, E. B. *J. Org. Chem.* **1986**, *51*, 4784.
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(9) (a) Lin, A. J.; Cosby, L. A.; Shansky, C. W.; Sartorelli, A. C. *J. Med. Chem.* **1972**, *15*, 1247. (b) Lin, A. J.; Pardini, R. S.; Cosby, L. A.; Lillis, B. J.; Shansky, C. W.; Sartorelli, A. J. *J. Med. Chem.* **1973**, *16*, 1268. (c) Lin, A. J.; Lillis, B. J.; Sartorelli, A. C. *J. Med. Chem.* **1975**, *18*, 917. (d) Lin, A. J.; Shansky, C. W.; Sartorelli, A. C. *J. Med. Chem.* **1974**, *17*, 558. (e) Lin, A. J.; Sartorelli, A. C. *J. Med. Chem.* **1976**, *19*, 1336. (f) Lin, T.-S.; Teicher, B. A.; Sartorelli, A. C. *J. Med. Chem.* **1980**, *23*, 1237.
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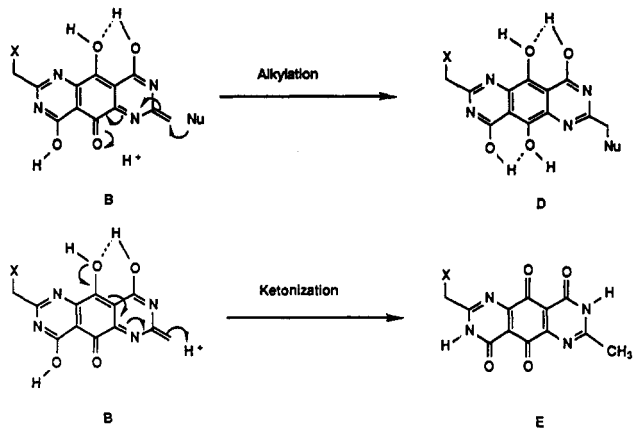
0022-3263/92/1957-5649$03.00/0

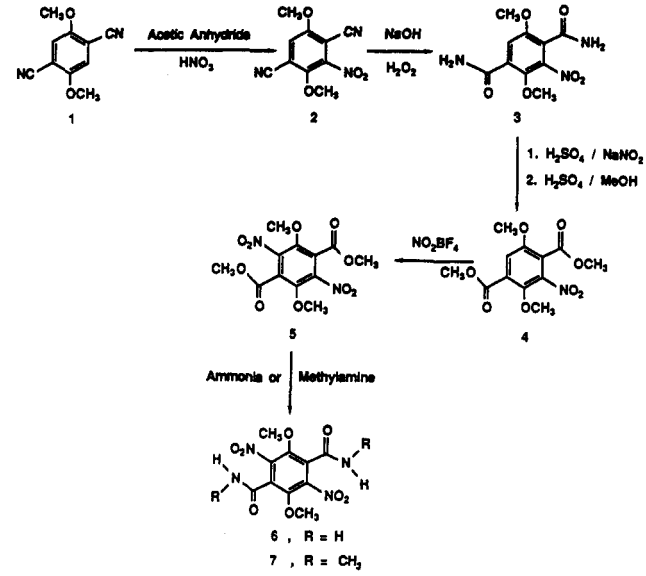
Chart III



designed as alkylating agents utilizing an S_N1 mechanism. Shown in Chart II is quinone methide (**B**) formation from a reduced tetrone derivative wherein R = H (**A**). The choice of the pyrimido[4,5-*g*]quinazoline ring system for study is based on its anthraquinone-like features; compare the structure of the pyrimido[4,5-*g*]quinazoline quinone methide (**B**) with that of an anthracycline quinone methide (**C**) in Chart II. The anthraquinone pharmacophore is present in many diverse types of DNA-directed antitumor agents including the anthracyclines,[11] mitoxanthrone,[12] and dynemicin.[13] It was proposed that pyrimido[4,5-*g*]-quinazoline alkylating agents would interact with DNA in a manner similar to anthraquinones and thereby alkylate DNA residues. Preliminary cancer cell cytotoxicity studies presented herein indicate that the tetrone derivatives in Chart I are inactive and that the dione derivatives are very cytotoxic. It is not yet known, however, whether or not cytotoxicity pertains to the anthraquinone-like structure of the pyrimido[4,5-*g*]quinazoline ring.

Physical studies described in this report answered questions concerning quinone methide formation and fate. One query concerned the influence of structure on quinone methide fate. In a previous study, we postulated that the rate of quinone methide formation increases as the reduction potential of the quinone/hydroquinone couple decreases.[6] In the present report we show that a linear free energy relationship exists between the rate of quinone methide formation and the reduction potential for structurally diverse systems. Another query concerned the structural features which influence the competition between ketonization and alkylation by the quinone methide, Chart III. Previous studies suggest that this competition is under thermodynamic control.[5,6] Thus, factors which stabilize the hydroquinone product favor alkylation (nucleophile trapping); conversely, factors which stabilize the quinone product favor ketonization (electrophile trapping). In the present report, we show that it is possible to control quinone methide fate by varying the substituents at the N(3) and N(8) positions. When the substituent is H (R = H in the tetrone derivative of Chart I), the quinone methide can trap nucleophiles since the hydroquinone product is stabilized by internal hydrogen bonds (**D** in Chart III).[14] However, when R = CH_3, there are no internal hydrogen bonds and the quinone methide traps electrophiles to afford the relatively stable quinone species.

Scheme I



The stabilizing influence of internal hydrogen bonds on a pyrimido[4,5-*g*]quinazoline hydroquinone (**A** in Chart II) prompted us to carry out a comparative study of aerobic hydroquinone oxidation rates for systems with R = H and CH_3. The system with internal hydrogen bonds (R = H) oxidized with a rate constant 2 orders of magnitude slower than that of the system without such bonds (R = CH_3). This finding may have a bearing on future designs of low cardiotoxicity antitumor agents.

Results and Discussion

Synthesis. There are few reports of pyrimido[4,5-*g*]-quinazolines in the literature. Although simple analogues were prepared after the turn of the century,[15] this laboratory reported the first quinone derivatives in 1988.[14] The strategy employed for the synthesis of pyrimido[4,5-*g*]-quinazoline-4,5,9,10-tetrones (quinones) without leaving group centers (e.g., methoxymethyls or halomethyls) was to annelate both pyrimidinone rings to a nitrobenzene derivative.[14] The nitro group was then reduced to the amine, from which the benzoquinone ring was synthesized by dichromate oxidation. In the presence of a leaving group center, dichromate treatment results in oxidation of this center (e.g., methoxymethyl to carboxylic acid). Thus, the pyrimido[4,5-*g*]quinazoline-based reductive alkylating agents were prepared by annelating the pyrimidinone rings to a *p*-dimethoxybenzene derivative. The *p*-dimethoxy derivative was then readily converted to the desired quinone or hydroquinone derivative. The preparation of pyrimido[4,5-*g*]quinazoline reductive alkylating agents is described in Schemes I–V.

Scheme I shows the preparation of the hexasubstituted benzene derivatives **6** and **7**, which were starting materials for all pyrimido[4,5-*g*]quinazolines described herein. The nitration of **1** only afforded the mononitrated product **2**,

(11) (a) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 2380. (b) Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 2504. (c) Ramakrishnan, K.; Fisher, J. *J. Am. Chem. Soc.* **1983**, *105*, 7187. (d) Abdella, B. R. J.; Fisher, J. *Environ. Health Perspect.* **1985**, *64*, 3.

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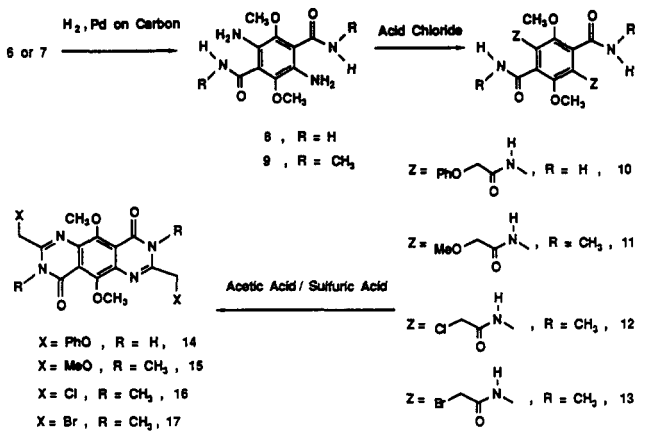
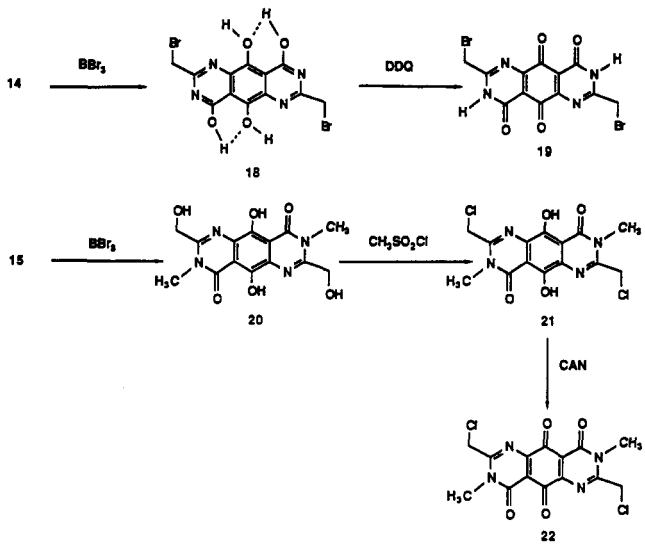
Scheme II



Scheme III



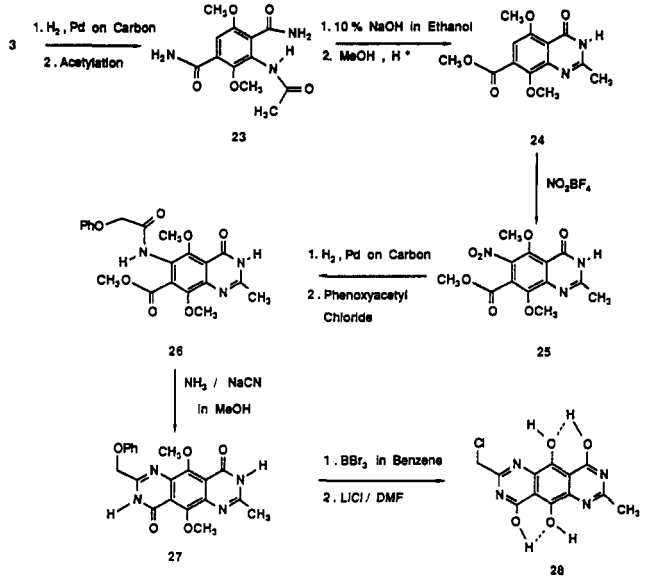
but the second nitration was possible after conversion of the nitrile groups to esters. Although nitration para to a nitro group would appear difficult, the conversion of **4** to **5** occurs under mild conditions. Perhaps the steric bulk of the esters forces the nitro group of **4** out of plane with the benzene ring thereby removing its resonance electron-withdrawing capability.

Annelation of the pyrimidinone ring to **6** or **7**, as shown in Scheme II, afforded the pyrimido[4,5-*g*]quinazolinedione derivatives **14**–**17**.

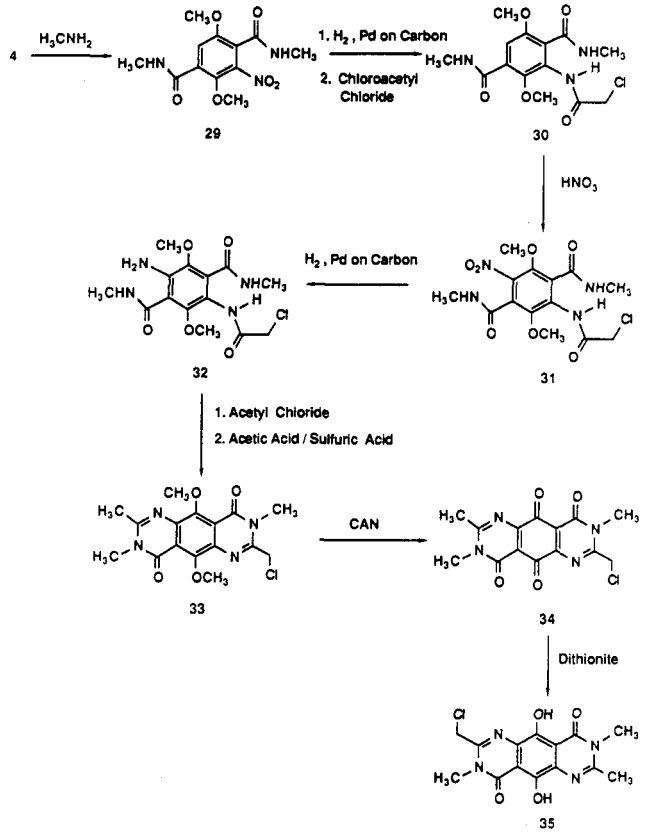
Compounds **19** and **22**, the tetrones shown in Chart I, were prepared as illustrated in Scheme III. In the case of the N-unsubstituted derivative **14**, O-demethylation and dephenoxylation to afford **18** were readily carried out by BBr_3 treatment. The steric bulk of the *N*-methyl substituents required the use of the methoxymethyl derivative **15** rather than the phenoxymethyl derivative, however. In the phenoxymethyl analogue of **15**, no dephenoxylation occurs on treatment with BBr_3 since bromide cannot attack the sterically hindered methylene centers. In **15**, bromide attack occurs only at the methyl centers to afford **20** as the product. Methanesulfonyl chloride treatment of **20** provided the desired chloromethyl derivative **21**, which was oxidized to **22** with ceric ammonium nitrate (CAN).

Schemes IV and V describe the preparation of N-unsubstituted and N-methylated hydroquinone analogues bearing only one leaving group, **28** and **35**, respectively. The hydrolysis of these hydroquinones was studied in order to gain mechanistic insights into quinone methide formation and fate. The synthetic steps in Schemes IV and V are straightforward, but the conditions for the final steps (**27** → **28** and **34** → **35**) are noteworthy. Hydroquinone **28** is stabilized by internal hydrogen bonds, as shown in Scheme IV, and thus the demethylation/dephenoxylation and halide exchange steps smoothly convert **27** to **28**. Hydroquinone **35**, on the other hand, is not stabilized by internal hydrogen bonds and is therefore very reactive. This hydroquinone rapidly eliminates HCl in any protic solvent to afford the quinone methide, which ketonizes to the tetramethyl derivative (vide infra). The stable quinone **34** was converted to **35** in chloroform solvent by shaking with aqueous dithionite for 30 s. The hydrolytically labile **35** was isolated from the chloroform layer in sufficiently pure form for our kinetic studies.

Scheme IV



Scheme V



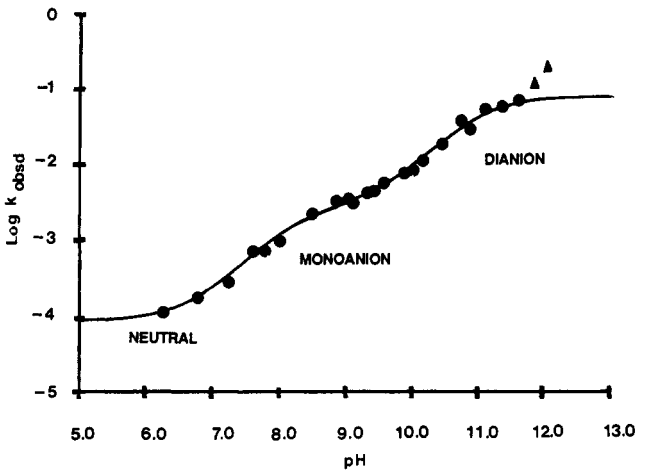


Figure 1. pH–rate profile for the conversion of neutral **28** and its anionic forms to quinone methide and carbocation species. Rates were measured in anaerobic buffer at 30.0 ± 0.2 °C with μ = 1.0, KCl. The two high pH points (▲) are not included in the computer fit.

Internal Hydrogen Bond Formation. Fused pyrimidone rings usually exist in aqueous solution as the oxo tautomers (e.g., compound **35**).[16] Studies in this laboratory revealed that the fused pyrimidone rings of N-unsubstituted pyrimido[4,5-*g*]quinazoline hydroquinones exist in aqueous solution as the enol tautomers (e.g., compound **28**).[14] The driving force for the enolization is the formation of internal hydrogen bonds to the hydroquinone hydroxyl groups. Verification of the presence of internal hydrogen bonds was possible employing UV–vis spectral studies, electrochemical studies, and pK_a measurements.[14] Internal hydrogen bonds stabilize the pyrimido[4,5-*g*]quinazoline hydroquinone resulting in high quinone reduction potentials (each hydrogen bond raises the quinone reduction potential 65 mV [NHE]). In contrast, the N-methylated analogue, which cannot form internal hydrogen bonds, possesses relatively low quinone reduction potentials. The pK_a value for acid dissociation from the hydroquinone hydroxyl group is also greatly affected by internal hydrogen bonding, which stabilizes the hydroxyl anion (pK_a of 8.2 with hydrogen bonding and a pK_a of 10.3 without).

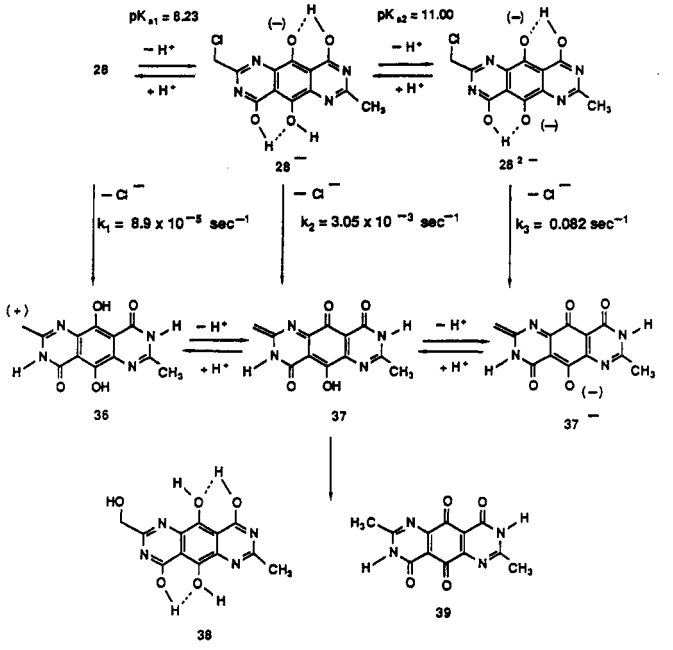
The findings noted above led to the postulate that internal hydrogen bonds can control quinone methide formation and fate by influencing hydroquinone stability.

Quinone Methide Chemistry. Insights into the formation and fate of pyrimidoquinazoline quinone methides were obtained from hydrolytic studies of hydroquinones **28** and **35**. Both hydroquinones eliminate the chloride leaving group to afford a reactive quinone methide species capable of trapping nucleophiles and electrophiles. Details of quinone methide formation and fate were obtained from pH–rate profiles and from product studies.

The hydrolysis of **28** was followed spectrophotometrically at 430 nm in anaerobic aqueous buffer over a pH range of 6–12 with [**28**] = 5 × 10^{-5} M. Absorbance vs time plots for hydrolysis are first order in character, with the first-order rate constants (k_{obsd}) independent of buffer concentration but dependent on pH as shown in Figure 1. These first-order rate constants are dependent on the halide leaving group (Br or Cl), indicating that leaving-group elimination occurs in the rate-determining step. The use of known hydroquinone and quinone extinction

(16) (a) Hearn, J. M.; Morton, R. A.; Simpson, J. C. E. *J. Chem. Soc.* **1951**, 3318. (b) Armarego, W. L. F. In *Fused Pyrimidines, Part 1, Quinazolines*; Brown, D. J.; Ed.; Interscience: New York, 1967; pp 102–104.

Scheme VI



coefficients[14] at 430 nm permitted the calculation of product yields: 69 ± 1% **38** and 31 ± 1% **39** regardless of the pH (product structures shown in Scheme VI). The accuracy of the yield determinations was enhanced by the intense hydroquinone visible spectrum and the absence of visible absorbances for the quinone derivative.[14] Product isolation studies confirmed the presence of **39**, but the polar hydroquinone product **38** could not be isolated (see Experimental Section). The presence of **38** is postulated based on previous studies, which demonstrated water trapping by quinone methide species.

The mechanism of **38** and **39** formation from **28** is discussed in conjunction with Scheme VI. The pH-rate data in Figure 1 indicate that rate-determining chloride elimination occurs from the neutral, monoanion, and dianion forms of **28**. Plateaus corresponding to the three eliminations are indicated on Figure 1. The monoanion and dianion plateaus are only inflections rather than the classic +1 to 0 slope change seen in pH–rate profiles. Consideration of the presence of inflections is required from the data, which cannot be fit to a simple base catalysis mechanism (slope = +1). Also, direct evidence of monoanion formation was obtained from spectrophotometric titration, pK_a = 8.26 ± 0.15 (see ref 14 for experimental details). The increasing rates above pH 11 may pertain to equilibrium trianion formation and rate-determining elimination of chloride from the trianion.

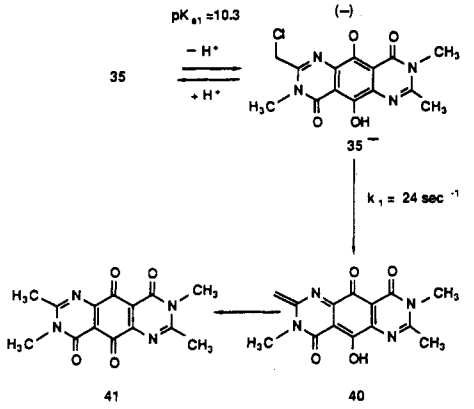
Consideration of rate-determining chloride elimination from the three forms of **28** (**28**, **28**$^-$, and **28**$^{2-}$) provides the rate law shown in eq 1 where the rate and equilibrium

$$k_{obsd} = \frac{k_1 a_H^2 + k_2 a_H K_{a1} + k_3 K_{a1} K_{a2}}{a_H^2 + a_H K_{a1} + K_{a1} K_{a2}} \quad (1)$$

constants are those shown in Scheme VI. The contribution of chloride elimination from the trianion to k_{obsd} is not considered in eq 1.

The numerical values for the rate and equilibrium constants (shown in Scheme VI) were obtained by computer-fitting the data in Figure 1 to eq 1. The solid line in Figure 1 was then generated from eq 1 substituted with these constants. The independently determined pK_a for monoanion formation (8.26 ± 0.15)[14] is in agreement with the kinetic pK_a value (8.23). This observation suggests that

Scheme VII



nonprotic equilibria are not an important part of the mechanism. The presence of such equilibria could result in a significant difference between the kinetically-determined pK_a value and the actual value obtained by titration.[17]

Chloride elimination from the neutral and anionic forms of **28** affords an equilibrating mixture of protonated (**36**), neutral (**37**), and anionic (**37**$^-$) quinone methides. Only the oxo tautomers of the quinone methides are shown in Scheme VI, although it is possible these species can form the enol tautomers. Proton trapping by **37**$^-$, in competition with nucleophile trapping of **37**, then affords a mixture of **38** and **39**. This mechanism explains the observed pH independence of the ratio [**38**]/[**39**]. Thus, proton trapping of **37**$^-$ and water trapping of **37** both possess transition states wherein the net charge is zero. Similar pH independence of product ratios was also seen in the benzimidazolequinone methides.[3] At very low pH values, the carbocation **36** will probably be present in significant quantities and nucleophile trapping to afford **38** will predominate. In the pH range studied (>6), **36** probably dissociates completely to **37** and **37**$^-$.

The hydrolysis of **35** in anaerobic buffer proceeds by a first-order process to afford **41** as the sole product. In contrast to **28**, the hydrolysis of **35** occurs at stopped-flow rates much above pH 8. The pH–rate profile, obtained over the pH range of 5–8, is a straight line of slope +1. This profile is attributed to equilibrium hydroxyl anion formation (**35** $\rightleftarrows$ **35**$^-$ + H$^+$, pK_a = 10.3) and loss of chloride under the condition of pH < 10.3, Scheme VII. Computer fitting the pH–rate data to $k_{obsd} = k_1K_{a1}/a_H$, and substituting pK_{a1} = 10.3, provided an elimination rate constant of k_1 = 24 s^{-1}. The value of 10.3 for the pK_a was obtained by spectrophotometric titration of an analogue of **35**, wherein chloromethyl is methyl, under strict anaerobic conditions.[14]

The Influence of Internal Hydrogen Bonding on Quinone Methide Formation and Fate. Our previous studies of pyrimido[4,5-g]quinazolines documented the presence of internal hydrogen bonds in these systems (see Chart II) by means of UV–vis spectral comparisons, pK_a measurements, and electrochemical measurements.[14] The influence of internal hydrogen bonds on quinone methide formation is apparent from the hydrolysis studies discussed above. Internal hydrogen bonding lowers the free energy of **28** and its anionic forms resulting in slow chloride eliminations, Scheme VI. In contrast, **35** cannot form internal hydrogen bonds and the elimination of chloride occurs with facility (compare $k_1 = 3.05 \times 10^{-3}$ s^{-1} for elimination of chloride from **28**$^-$ with 24 s^{-1} for the same elimination from **35**$^-$). Internal hydrogen bonding also influences the quinone methide trapping products: the quinone methide species from **28** traps nucleophiles and protons (ratio of 69:31) while the quinone methide from **35** traps only protons. Previous work in this laboratory showed that quinone methide trapping is under thermodynamic control.[6] Stabilization of the hydroquinone by internal hydrogen bonding favors nucleophile trapping since a hydroquinone (**38**) is the product. Without internal hydrogen bonds, electrophile trapping is favored since a stable quinone (**41**) is the product.

(17) Schmir, G. L.; Bruice, T. C. *J. Am. Chem. Soc.* **1959**, *81*, 4552.

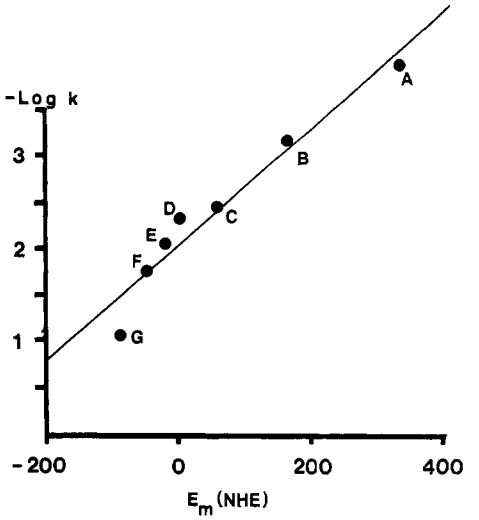
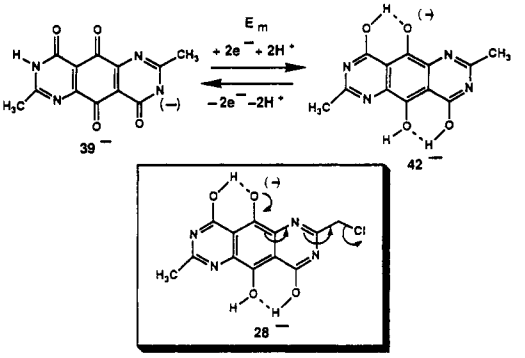


Figure 2. Linear free energy relationship for the quinone methide forming reactions in Table I. The *y*-axis is the log of the rate of quinone methide formation and the *x*-axis is the quinone reduction potential measured against the normal hydrogen electrode.

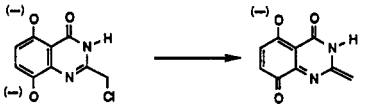
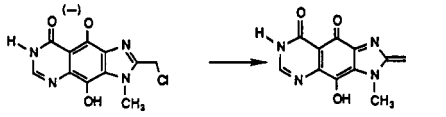
Scheme VIII



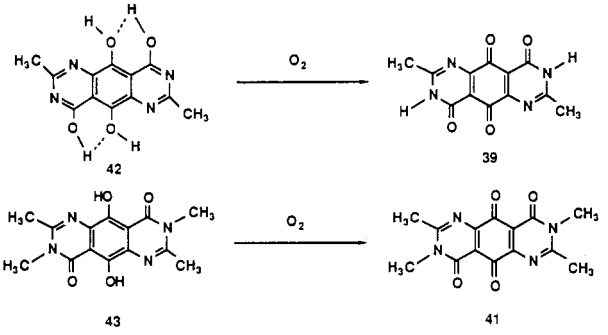
Linear Free Energy Relationship for Quinone Methide Formation. This relationship was obtained by plotting the log of first-order rate constants for quinone methide formation vs two-electron quinone reduction potentials, Figure 2. The quinone reduction potential is a measure of the free energy difference between the quinone and hydroquinone forms of the redox couple, and thus it can be used as an indirect measure of hydroquinone free energy. Data points for the linear free energy plot were obtained as discussed below in conjunction with Scheme VIII.

Shown in the inset of Scheme VIII is an elimination reaction from **28**$^-$ to afford a quinone methide species. The first-order rate constant for this reaction was obtained from the pH–rate data in Figure 1. The corresponding reduction potential, **39**$^-$/**42**$^-$, was obtained from a previously published Nernst Clark plot.[14] All the quinone reduction potentials in the linear free energy plot were obtained with quinone systems without the leaving group present. The reduction potential is read off a Nernst Clark Plot at a pH

Table I. Quinone Methide Forming Reactions

entry	reaction	ref
A	28 → 36	This Study
B		8a
C	28⁻ → 37	This Study
D		15
E		8a
F		8b
G	28²⁻ → 37⁻	This Study

Scheme IX



value where the particular hydroquinone species (neutral, monoanion, or dianion) is reversibly formed.

The reactions used to obtain the plot in Figure 2 are found in Table I. There is an excellent correlation of log k with the quinone reduction potential, even though the reactivity range spans 0.5 V and a structural diversity of quinone methides are included. It is apparent from the excellent correlation that all the reactions involve an elimination of the leaving group in the rate-determining step. A substitution reaction, such as the conversion of **28** to **38**, must necessarily involve elmination of the leaving group followed by nucleophile trapping of the intermediate, and not an S_N2-type process.

Oxygen Reactivity. In order to assess the capability of the title systems to generate reactive oxygen species by quinone/hydroquinone cycling, the rate laws were determined for oxygen oxidation of **42** and **43**, Scheme IX. These respective hydroquinones are analogues of **28** and **35** without leaving groups present. As expected, the internal hydrogen bonds of **42** slow oxidation at high pH while the absence of hydrogen bonds in **43** result in stopped flow oxidations under these conditions. However, the crossing of the pH–rate profiles near neutrality (Figure 3) results in similar rates of oxidation for both systems at physiological pH.

In air saturated aqueous buffers, both **42** and **43** are converted to the corresponding quinones (Scheme IX) by pseudo-first-order processes. Inspection of the pH–rate

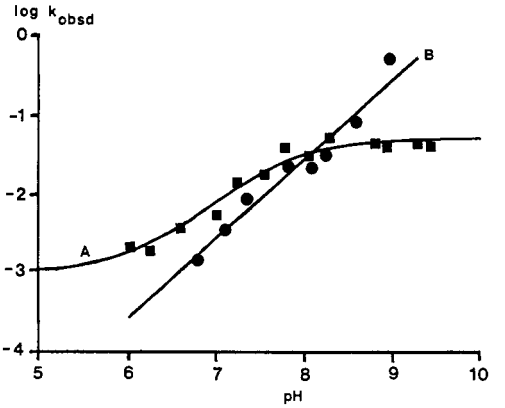


Figure 3. pH-rate profiles or air oxidation of hydroquinones **42** (plot A) and **43** (plot B) in aerobic aqueous buffer at 30.0 ± 0.2 °C with μ = 1.0 (KCl).

profile for the oxidation of **42**, plot A in Figure 3, reveals that both the neutral and monoanion forms of this hydroquinone are oxidized to quinone **39**. The solid curve of plot A was generated from the rate law, $k_{obsd} = (k_1a_H + k_2K_{a1})/(a_H + K_{a1})$, where k_1 is the pseudo-first-order rate of oxidation of the neutral species (1×10^{-3} s^{-1}) by molecular O_2, k_2 is the pseudo-first-order rate of oxidation of the hydroquinone monoanion (0.046 s^{-1}) by molecular O_2, K_{a1} is the acid dissociation constant (pK_{a1} = 7.78), and a_H is the proton activity determined with a pH meter. The acid dissociation constant obtained from the pH–rate data is nearly the same as the value determined previously, 8.26 ± 0.15.[14]

In contrast to **42**, the oxidation of **43** to **41** occurs at stopped-flow rates at or above pH 9. The pH–rate profile possesses a slope of one, plot B of Figure 3, which may reflect equilibrium hydroquinone monoanion formation followed by oxidation. If this is the case, the monoanion of **43** oxidizes to **41** at 5.8 s^{-1} (based on the pK_a of 10.3[14] for **43**).

From the foregoing results, it is apparent that internal hydrogen bonding interactions can substantially slow the oxidation of a hydroquinone monoanion. Thus, **42**$^-$ oxidizes 128-fold slower than **43**$^-$ in aerobic buffer. The crossing of the pH–rate profiles in Figure 3 results in similar rates of oxidation for both systems at physiological pH: **42** oxidizes at 1.7×10^{-2} s^{-1} while **43** oxidizes at 9×10^{-3} s^{-1}. Both hydroquinone systems are still more stable toward oxygen than reduced anthracyclines, which rapidly oxidize on mixing with air.[18]

Cytotoxicity Studies. We anticipated that the pyrimido[4,5-*g*]quinazoline **19** would possess cytotoxic activity since this system affords an alkylating quinone methide species upon reduction and its tricyclic structure resembles anthracyclines and other antitumor agents.[19] Screening studies[20] against ht-29 and WiDr colon cancer revealed that **19** actually possesses poor activity, $IC_{50} > 10^{-6}$ M (IC_{50} is the concentration needed to inhibit 50% of treated cells). Likewise, the methylated analogue **22** also possesses poor cytotoxic activity.

Assays of **16** and **17** against colon, breast, and ovarian cancer cell lines, on the other hand, revealed excellent cytotoxic activity, IC_{50} values as low as 20 nM. Preliminary studies suggest that **16** and **17** act as a bis-alkylating agent

(18) Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1990**, *112*, 9423.
(19) (a) Berman, H. M.; Young, P. R. *Ann. Rev. Biophys. Bioeng.* **1981**, *10*, 87. (b) Wilson, W. D.; Jones, R. L. *Adv. Pharmacol. Chemother.* **1981**, *18*, 177. (c) Müller, W.; Crothers, D. M. *Eur. J. Biochem.* **1975**, *54*, 267.
(20) Studies carried out in the laboratory of Professor David S. Alberts, Arizona Cancer Center, Tucson, AZ.

(both alkylating centers are necessary for activity) and that the methoxy groups are also necessary for activity. The latter observation is consistent with an S_N1 alkylation mechanism, which requires resonance electron donation by the methoxy groups to stabilize the carbocation.

Details of the structure–activity relationship for the pyrimido[4,5-g]quinazoline cytotoxic agents, and of the cytotoxicity mechanism of these agents, will be presented in future publications.

Conclusion

The synthesis of pyrimido[4,5-g]quinazolines functionalized as reductive alkylating agents was carried out starting with hexasubstituted benzene derivatives. Kinetic studies of the reductive alkylation process revealed that quinone methides based on the pyrimido[4,5-g]quinazoline ring system can form in solution and trap nucleophiles and/or electrophiles. By means of a linear free energy relationship (LFER), we show that quinone reduction potential can be used to predict the rate of quinone methide formation for structurally diverse systems. The LFER currently spans a 500-mV potential range. Efforts are underway to extend the range of this plot further.

Insights were also obtained into the factors which influence electrophile and nucleophilic trapping by the quinone methide species. The trapping process is under thermodynamic control: stabilizing the hydroquinone results in nucleophile trapping and stabilizing the quinone results in electrophile trapping. Pyrimido[4,5-g]quinazoline quinone methides, wherein R = H, are nucleophile traps due to hydroquinone stabilization by internal hydrogen bonds. In contrast, the pyrimido[4,5-g]quinazoline quinone methide, wherein R = CH_3, only traps protons.

Internal hydrogen bonds in the pyrimido[4,5-g]quinazoline hydroquinone, wherein R = H, slow the rate of aerobic oxidation to the quinone. This finding may be important in the design of low cardiotoxicity quinone antitumor agents. Anthracycline antitumor agents produce cardiotoxic oxygen radicals by cycling between the hydroquinone and quinone forms with the aid of nonspecific quinone reductases and oxygen.[21] The slow reoxidation rates of pyrimido[4,5-g]quinazoline hydroquinones will decrease oxygen radical generation which in turn may reduce cardiotoxicity.

The cancer cell cytotoxicity results presented in this report, along with physical studies, reflect ongoing efforts in this laboratory to design alkylating drugs by considering quinone methide and carbocation reactivity and structure. The pyrimido[4,5-g]quinazolines **16** and **17** exhibit cytotoxicity probably due to structure, lipophilicity, and the formation of a carbocation stabilized by the methoxy substituents capable of acting as a selective nucleophile trap.[22] Ongoing structure–activity studies of pyrimido[4,5-g]quinazoline antitumor agents will provide further insights into the importance of structure, lipophilicity, and reactivity.

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 (hydroquinones) were not heated above room temperature. Some of the 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 was run with silica gel plates with fluorescent indicator employing a variety of solvents. IR spectra were taken as KBr pellets or thin films; the strongest IR absorbances are reported. ^1H NMR spectra were obtained on a 400-MHz spectrometer, and chemical shifts are reported relative to TMS.

Kinetic Studies of Hydrolysis. The hydrolytic studies of **28** and **35** were carried out in anaerobic aqueous buffer held at 30.0 ± 0.2 °C employing Thunberg cuvettes as previously described.[23] Details of the hydrolysis studies are provided below.

A dimethyl sulfoxide stock of the compound to be studied was prepared fresh, and 50 μL of this stock was added to the top port of a Thunberg cuvette. To the bottom port there was added 2.95 mL of aqueous buffer, μ = 1.0 (KCl). The Thunberg was sealed (with Apiezon N grease), and the contents of the top and bottom ports were deoxygenated by passing argon (filtered through an Oxiclear filter and then humidified) into the solutions through thin Teflon tubes for 30 min. The tubes were then removed, and the Thunberg cuvette was closed. After equilibration at 30 ± 0.2 °C, the contents were mixed and the increase in absorbance followed at 410 nm (pH of buffers <8) or 430 nm (pH of buffers >8). The absorbance vs time data were fit to a first-order rate law.

The presence of buffer catalysis was determined by 10-fold buffer dilutions at constant pH (±0.03 pH units). There was no buffer catalysis over the pH range studied. The presence of second-order reactions (redox reaction involving quinone product and hydroquinone starting material) was assessed by measuring first-order rate constants over a 5-fold dilution of hydroquinone starting material. The first-order character of the absorbances vs time plots and the calculated k_{obsd} values were unaffected by dilution until concentrations exceeded 1×10^{-4} M.

pH–rate equations for hydroquinone hydrolysis and hydroquinone reoxidation were derived from material balance and the acid–base equilibria involved in the mechanism.[24] The rate equation for the mechanism in Scheme VI (eq 1) was derived considering $[28_T] = [28] + [28^-] + [28^{2-}]$ and the first-order elimination of chloride from each of the three forms of **28**.

Hydrolysis Products of 28. To 250 mL of pH 11.15 KOH buffer was added a solution of 18.6 mg of **28** in 3 mL of dimethyl sulfoxide under strict anaerobic conditions. After a 30-min reaction time, air was introduced into the reaction mixture resulting in oxidation of the hydroquinone product. The reaction volume was reduced to 50 mL in vacuo without heating above room temperature. TLC (*n*-butanol/acetic acid/water (5:2:3)) of the concentrated mixture revealed the presence of quinone **39** (see ref 14 for physical properties) and another quinone, presumably the air oxidation product of the hydroquinone hydrolysis product **38**.

The concentrated mixture was placed on a 50-mL Dowex 1-X2 100–200-mesh chloride column. After eluting the column with water (~10 volumes), the quinone product was eluted with concentrated hydrochloric acid. Evaporation of the product fractions afforded a yellow solid, identified by ^1H NMR as pure **39**, in very low yield. The oxidized form of **38** could not be isolated.

Spectral studies of completed reaction mixtures provided further insights into the identity of the hydrolysis products as well as the product ratio as a function of pH. At three pH values (5.92, 9.04, and 11.17) 6×10^{-5} M **28** was hydrolyzed in a Thunberg cuvette under an argon atmosphere at 30.0 ± 0.2 °C. After 9 half-lives (followed at 430 nm), a UV–vis spectrum was run from 250 to 600 nm. The Thunberg was opened to the air resulting in oxidation of hydroquinone present. After oxidation was complete (assessed by following Δ absorbance with time at 430 nm), another UV–vis spectrum was run from 250 to 600 nm. The spectrum of the oxidized reaction was typical of a pyrimido[4,5-g]quinazolinequinone, whereas the reduced spectrum had λ_{max} values typical of both quinone and hydroquinone derivatives of pyrimido[4,5-g]quinazolines (see ref 14 for UV–vis spectra). The relative amounts of quinone and hydroquinone products were

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

(22) Pross, A. *Adv. Phys. Org. Chem.* **1977**, *14*, 69. See Figure 8, the selectivity of a carbocation for a nucleophile increases with its stability.

(23) Skibo, E. B.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 3304.

(24) For an example of a pH-rate law calculation see: Bruice, T. C.; Benkovic, S. J. *Bioorganic Mechanisms*, W. A. Benjamin: New York, 1966; Vol. 1, pp 11–16.

determined from the reduced and oxidized spectra and from extinction coefficients determined at each of the three pH values employing pure pyrimido[4,5-g]quinazolinequinone and hydroquinone derivatives.

Hydrolysis of 35 afforded **41** in quantitative yield, based on UV–vis spectra of completed reactions (see ref 14 for spectra of **41**). The preparative hydrolysis of **35** was carried out in 250 mL of anaerobic buffer (water or aqueous buffer held at any pH could be used). The completed reaction was extracted with chloroform to remove **41**. Drying the extract (Na_2SO_4), evaporation to a small volume, and then crystallization by adding hexane afforded pure **41**.

Air Oxidation of 42 and 43. Hydroquinones **42** and **43** were prepared as previously described.[14] A hydroquinone solution in dimethyl sulfoxide (50 μL) was added to 2.95 mL of aerobic aqueous buffer (μ = 1.0, KCl) at 30.0 ± 0.2 °C. The disappearance of hydroquinone was monitored at 410 nm. Absorbance vs time plots were first order in character. The oxidation products of **42** and **43** (**39** and **40**, respectively) were obtained in quantitative yield (by UV–vis product spectra measurement).

Synthesis and physical properties of new compounds are provided below.

3,6-Dicyano-1,4-dimethoxy-2-nitrobenzene (2). A suspension consisting of 30 mL of acetic anhydride and 995 mg (5.29 mmol) of **1**[25] was chilled to 0 °C. To the chilled suspension was added 15 mL of 90% nitric acid portionwise so as to maintain a reaction temperature less than 20 °C. After the addition was completed, the reaction was left to stir at room temperature for 15 min and then poured over ~500 g of crushed ice. The resulting yellow precipitate was filtered off and washed with cold water. The solid was recrystallized by dissolution in a minimum amount of chloroform followed by addition of hexane: 870-mg (71%) yield; mp 146–147 °C; TLC (ethyl acetate) R_f = 0.61; IR (KBr pellet) 2239, 1552, 1490, 1403, 1365, 1276, 1249, 1041, 962, 925 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 8.24 (1 H, s, 5-H), 4.04 (6 H, s, methoxys); mass spectrum (EI) m/z 233 (M^+). Anal. Calcd for $C_{10}H_7N_3O_4 \cdot 0.25H_2O$: C, 50.53; H, 3.18; N, 17.67. Found: C, 50.98; H, 2.95; N, 17.61.

3,6-Dicarbamyl-1,4-dimethoxy-2-nitrobenzene (3). To 508 mg (2.18 mmol) of **2** in 10 mL of ethanol, preheated to 40 °C, was added 5 mL of 1 N NaOH and then 5 mL of 10% hydrogen peroxide. After a reaction time of ~5 min, a yellow solid crystallized from solution. After being heated for an additional 15 min, the solution was poured over 50 mL of ice–water. The solid which resulted was filtered and washed with water and then ethanol to afford the product as a pure white-colored solid: 470-mg (80%) yield; mp 288–290 °C; TLC (chloroform/methanol (9:1)) R_f = 0.23; IR (KBr pellet) 3374, 3186, 1657, 1627, 1535, 1482, 1473, 1423, 1237, 1031 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 7.97 and 7.84 (4 H, 2 br s, 3- and 6-carbamyls, no assignments made), 7.39 (1 H, s, 5-H), 3.87 and 3.78 (6 H, 2 s, 1- and 4-methoxys, no assignments made); mass spectrum (EI mode) m/z 269 (M^+). Anal. Calcd for $C_{10}H_{11}N_3O_6$: C, 44.62; H, 4.12; N, 15.61. Found: C, 44.45; H, 4.02; N, 15.47.

1,4-Dimethoxy-2-nitrobenzene-3,6-dicarboxylic acid dimethyl ester (4) was prepared by the following two-step procedure. To 100 mL of concentrated sulfuric acid was added 3.0 g (11 mmol) of **3** at room temperature. The reaction mixture was stirred at this temperature until the solid was fully dissolved, and then the mixture was chilled to 0 °C. To the chilled reaction mixture was added a solution consisting of 6 g of sodium nitrite in 60 mL water portionwise below the surface of the solution while maintaining a reaction temperature below 25 °C. After complete addition, the reaction mixture was heated at 70 °C until effervescence had ceased and then poured over ~500 g of crushed ice. The solid was filtered and washed with water to afford the off-white dicarboxylic acid derivative: 2.2 g (73%) yield; TLC (2-propanol/water/ammonia (7:1:2)) R_f = 0.49; ^1H NMR (dimethyl-d_6 sulfoxide) δ 7.64 (1 H, s, 5-H), 3.90 and 3.82 (6 H, 2 s, 1- and 4-methoxys, no assignments made); mass spectrum (EI mode) m/z 271 (M^+).

To a solution of 4.0 g (0.015 mmol) of the product obtained above in 100 mL of dry methanol was added 5 mL of concentrated sulfuric acid and the resulting mixture refluxed until TLC showed the reaction to be complete. The solvent was evaporated in vacuo to afford ~25 mL of a yellow liquid. After the liquid was poured into 200 mL of ice–water, the resulting mixture was extracted with 3 × 100 mL portions of chloroform. The extracts were rinsed with 10% sodium carbonate and then with water. Drying (Na_2SO_4) the extracts and removal of the solvent in vacuo afforded a residue which crystallized as yellow flakes upon addition of hexane: 3.3-g (75%) yield; mp 91–92 °C; TLC (2-propanol/water/ammonia (7:1:2)) R_f = 0.83; IR (KBr pellet) 2957, 1734, 1536, 1487, 1450, 1254, 1233, 1141, 1096 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 7.73 (1 H, s, 5-H), 3.93, 3.92, 3.83 and 3.82 (12 H, 4 s, methoxys, no assignments made); mass spectrum (EI mode) m/z 299 (M^+). Anal. Calcd for $C_{12}H_{13}NO_8$: C, 48.17; H, 4.38; N, 4.68. Found: C, 47.82; H, 4.33; N, 4.77.

1,4-Dimethoxy-2,5-dinitrobenzene-3,6-dicarboxylic Acid Dimethyl Ester (5). To 5.0 g (0.017 mol) of **4** dissolved in 100 mL of dry acetonitrile was added NO_2BF_4 until no starting material was evident by TLC. The solvent was removed in vacuo and the oily residue treated with 100 mL of ice–water. The resulting mixture was extracted with 3 × 100 mL portions of ethyl acetate. The extracts were dried (Na_2SO_4) and evaporated in vacuo to a solid residue, which upon recrystallization from ethanol afforded **5** as yellowish-white needles: 3.8-g (66%) yield; mp 126–127 °C; TLC (chloroform) R_f = 0.52; IR (KBr pellet) 1746, 1548, 1480, 1439, 1388, 1361, 1291, 1197, 1150, 1027 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 3.93 and 3.90 (12 H, 2 s, methoxys, no assignments made); mass spectrum (EI) m/z 344 (M^+). Anal. Calcd for $C_{12}H_{12}N_2O_{10}$: C, 41.87; H, 3.51; N, 8.14. Found: C, 41.79; H, 3.25; N, 7.96.

3,6-Dicarbamyl-1,4-dimethoxy-2,5-dinitrobenzene (6). To 50 mL of saturated methanolic ammonia was added 704 mg (2.05 mmol) of **5** and the reaction mixture left to stir at room temperature for 12 h. After the solvents were evaporated, the solid residue was collected and washed with ethanol to give the desired compound as a white solid: 467-mg (73%) yield; 291–293 °C dec; TLC (2-propanol/water/ammonia (7:1:2) R_f = 0.78; IR (KBr pellet) 3444, 3424, 3313, 3191, 1674, 1660, 1542, 1402, 1020 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 8.42 and 8.23 (4 H, 2 s, 3 and 6 carbamyls, no assignments made), 3.87 (6 H, s, methoxys); mass spectrum (EI mode) m/z 314 (M^+). Anal. Calcd for $C_{10}H_{10}N_4O_8$: C, 38.25; H, 3.21; N, 17.83. Found: C, 38.65; H, 3.04; N, 17.53.

3,6-Bis(methylcarbamyl)-1,4-dimethoxy-2,5-dinitrobenzene (7). To a solution of methylamine in methanol (100 mL, 67% w/w), cooled to −78 °C, was added 4.0 g (0.012 mol) of **5**. After the solution was allowed to come to room temperature over a period of 1 h, the precipitated product was filtered off and rinsed with ethanol and then chloroform to afford the desired compound as a white solid: 2.9-g (73%) yield; 297–299 °C dec; IR (KBr) 3293, 1655, 1570, 1536, 1474, 1416, 1396, 1373, 1059, 998 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 8.99–8.94 (2 H, m, 3- and 6-carbamyl protons) 3.82 (6 H, s, methoxys), 2.77 (6 H, d, J = 3.3 Hz, 3- and 6-carbamyl methyls); mass spectrum (EI mode) m/z 342 (M^+). Anal. Calcd for $C_{12}H_{14}N_4O_8$: C, 42.11; H, 4.12; N, 16.37. Found: C, 42.32; H, 4.01; N, 15.76.

3,6-Dicarbamyl-1,4-dimethoxy-2,5-diaminobenzene (8). A mixture consisting of 561 mg (1.79 mmol) of **6**, 50 mg of 5% Pd/C, and 100 mL of methanol was shaken under 50 psi of H_2 for 4 h. Upon completion of the reduction, 10 mL of concentrated hydrochloric acid was added immediately to the reaction mixture with stirring. After the acidified mixture was filtered through Celite and washed with methanol, the solvents were evaporated in vacuo and the solid residue thoroughly dried. The **8·2 HCl** was purified by dissolution in a minimum amount of hot methanol followed by addition of ethyl acetate: 568-mg (97%) yield; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.37; ^1H NMR (dimethyl-d_6 sulfoxide) δ 7.93 and 7.92 (4 H, 2 br s, 3- and 6-carbamyls, no assignments made), 3.69 (6 H, s, methoxys); mass spectrum (EI mode) m/z 254 (M^+).

3,6-Bis(methylcarbamyl)-1,4-dimethoxy-2,5-diaminobenzene (9). A mixture consisting of 978 mg (2.84 mmol) of **7**, 100 mg of 5% Pd/C, and 100 mL of methanol was shaken under 50 psi of H_2 for 4 h. Upon completion the reduction, 10 mL of concentrated hydrochloric acid was added to the reaction mixture with stirring. After the solution was filtered through Celite and the filtrate washed with methanol, the solvent was removed in

(25) Nolan, W. E.; Baude, F. J. *J. Org. Chem.* **1966**, *31*, 3321.

vacuo and the residue dried for several hours. The dihydrochloride salt of **9** was recrystallized by dissolution in a minimum amount of hot methanol followed by cooling and then addition of ethyl acetate: 893-mg (88%) yield; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.44; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 8.40 (2 H, br s, 3- and 6-carbamyl proton), 3.64 (6 H, s, methoxys), 2.81 (6 H, d, J = 4.0 Hz, 3- and 6-carbamyl methyls); mass spectrum (EI mode) m/z 282 (M^+).

2,5-Bis(phenoxyacetamido)-3,6-dicarbamyl-1,4-dimethoxybenzene (10). To the 568 mg (1.76 mmol) of **8·2HCl**, suspended in 50 mL of dry benzene, were added 0.6 mL (4.34 mmol) phenoxyacetyl chloride and 2.4 mL (17 mmol) of dry triethylamine. The resulting mixture was stirred under a dry atmosphere at room temperature for 4 h. The solvents were evaporated in vacuo using minimal heating and the residue treated with water to precipitate **10**. The product was recrystallized from dimethylformamide and then washed with water: 699-mg (77%) yield; mp 272–274 °C; TLC (ethyl acetate/methanol (9:1)) R_f = 0.32; IR (KBr pellet) 3385, 3262, 1701, 1680, 1600, 1510, 1492, 1456, 1367, 1222 cm^{-1}; ^{1}H (dimethyl-d_6 sulfoxide) δ 9.17 (2 H, s, 2,5-acetamido NH), δ 7.62, 7.57, 7.49 and 7.25 (4 H, 4 br s, 3- and 6-carbamyls, no assignments made), 7.34 (4 H, t, J = 8.1 Hz, aromatic, no assignment), 7.0 (6 H, d, J = 8.5 Hz, aromatic, no assignments made), 4.61 (4 H, s, methylenes), 3.68 and 3.58 (6 H, 2 s, 1- and 4-methoxys, no assignments made); mass spectrum (EI mode) m/z 522 (M^+). Anal. Calcd for $C_{26}H_{26}N_4O_8 \cdot 0.1H_2O$: C, 59.56; H, 5.04; N, 10.69. Found: C, 59.54; H, 5.12; N, 11.06.

2,5-Bis(methoxyacetamido)-3,6-bis(methylcarbamyl)-1,4-dimethoxybenzene (11). To 1.0 g (2.82 mmol) of **9·2HCl**, dissolved in 50 mL of dry dimethylformamide, were added 0.57 mL (6.24 mmol) of methoxyacetyl chloride and 1.1 mL (13.6 mmol) of dry pyridine. The resulting mixture was stirred at room temperature for 4 h. After the dimethylformamide was removed in vacuo, the solids were collected, rinsed with water, and then rinsed with ethanol. Recrystallization was carried out from dimethylformamide: 942-mg (78%) yield; mp 275–276 °C; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.47; IR (KBr pellet) 3361, 1700, 1627, 1579, 1506, 1472, 1410, 1328, 1115, 1053 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 9.16 (2 H, s, 2,5-acetamido NH), 7.80 (2 H, br q, 3- and 6-carbamyl H), 3.96 (4 H, s, methylenes), 3.65 and 3.37 (12 H, 2 s, methoxys, no assignments made), 2.70 (6 H, d, J = 4.5 Hz, 3- and 6-carbamyl methyls); mass spectrum (EI mode) m/z 426 (M^+).

2,5-Bis(chloroacetamido)-3,6-bis(methylcarbamyl)-1,4-dimethoxybenzene (12). To 497 mg (1.40 mmol) of **9·2HCl**, dissolved in 15 mL of dry dimethylformamide, were added 510 μL (6.30 mmol) of pyridine and 250 μL (3.14 mmol) of chloroacetyl chloride. After the reaction mixture was stirred at room temperature for 6 h, the solids were filtered off, rinsed with water, and dried: 431-mg (71%) yield; mp 146–148 °C and then 317–319 °C after thermal cyclization; TLC (ethyl acetate/methanol (9:1)) R_f = 0.24; IR (KBr pellet) 3269, 1669, 1644, 1567, 1526, 1472, 1409, 1328, 1057 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 9.72 (2 H, s, 2,5-acetamido NH), 7.87 (2 H, br s, 3- and 6-carbamyl Hs), 4.22 (4 H, s, methylenes), 3.65 (6 H, s, methoxys), 2.70 (6 H, d, J = 4.6 Hz, 3- and 6-carbamyl methyls); mass spectrum (EI, solids probe) m/z 434 (M^+, $^{35}Cl^{35}Cl$), 436 (M^+, $^{37}Cl^{35}Cl$), 438 (M^+, $^{37}Cl^{37}Cl$).

2,5-Bis(bromoacetamido)-3,6-bis(methylcarbamyl)-1,4-dimethoxybenzene (13). To a solution of 503 mg (1.42 mmol) of **9·2HCl** in 15 mL of dry dimethylformamide were added sequentially 533 μL (6.59 mmol) of pyridine and 282 μL (3.42 mmol) of bromoacetyl chloride. After the mixture was allowed to stir at room temperature for 6 h, the precipitated produced was filtered off and rinsed with water and then rinsed with ethanol: 393-mg (53%) yield; mp 308–310 °C; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.58; IR (KBr pellet) 3380, 3278, 1681, 1638, 1551, 1472, 1411, 1325, 1238, 1047 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 9.72 (2 H, s, 2,5-acetamido NH), 7.87 (2 H, br s, 3- and 6-carbamyl proton), 4.22 (4 H, s, methylenes), 3.65 (6 H, s, methoxys), 2.70 (6 H, d, J = 4.6 Hz, 3- and 6-carbamyl methyl); mass spectrum (EI mode) m/z 522, 524 and 526 (M^+ for the ^{79}Br, ^{79}Br; ^{79}Br, ^{81}Br; ^{81}Br, ^{81}Br combinations, respectively, at an intensity ratio of 1:2:1).

2,7-Bis(phenoxymethyl)-5,10-dimethoxypyrimido[4,5-g]quinazoline-4,9(3*H*,8*H*)-dione (14). To a solution consisting of 40 mL of acetic acid and 4 mL of concentrated sulfuric acid was added 394 mg (0.753 mmol) of **10**. The resulting mixture was refluxed for 4 h. After the mixture was allowed to cool to room temperature, the grayish colored precipitate was collected, washed with acetic acid, and then washed with water to afford pure **14** as a yellow solid: 260-mg (71%) yield; 290 °C dec; TLC (ethyl acetate/methanol (9:1)) R_f = 0.56; IR (KBr pellet) 2967, 1687, 1630, 1599, 1492, 1463, 1427, 1239, 1206, 1066 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 7.32 (4 H, t, J = 7.4 Hz, aromatic, no assignments made), 7.08 (4 H, d, J = 7.8 Hz, aromatic, no assignments made), 6.98 (4 H, t, J = 7.4 Hz, aromatic, no assignments made), 5.02 (4 H, s, methylenes), 3.81 (6 H, s, methoxys); mass spectrum (EI mode) m/z 486 (M^+). Anal. Calcd for $C_{26}H_{22}N_4O_6$: C, 64.19; H, 4.56; N, 11.52. Found: C, 64.09; H, 4.52; N, 11.31.

2,7-Bis(methoxymethyl)-5,10-dimethoxy-3,8-dimethylpyrimido[4,5-g]quinazoline-4,9(3*H*,8*H*)-dione (15). To 100 mL of acetic acid and 4 mL of concentrated sulfuric acid was added 1.80 g (4.22 mmol) of **11**, and the mixture was heated at 100 °C for 5 h. After the solvents were evaporated down to ~5 mL, the oily residue was poured over 100 mL of ice–water and neutralized with sodium carbonate and the resulting solution extracted with 3 × 100-mL portions of chloroform. The combined extracts were evaporated to a small volume, placed on a silica column, and purified by flash chromatography employing ethyl acetate as the eluant. The product fraction was collected and evaporated to dryness. Recrystallization was carried out by redissolving the product in a minimum amount of chloroform followed by addition of hexane: 1.10-g (67%) yield; mp 223–224 °C; TLC (ethyl acetate/methanol (9:1)), R_f = 0.22; IR (KBr pellet) 1688, 1614, 1474, 1428, 1375, 1342, 1269, 1097, 1036, 797 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 4.58 (4 H, s, methylenes), 3.93, 3.62 and 3.42 (18 H, 3 × s, *N*- and *O*-methyls, no assignments made); mass spectrum (EI mode) m/z 390 (M^+). Anal. Calcd for $C_{18}H_{22}N_4O_8 \cdot 0.3H_2O$: C, 54.75; H, 5.74; N, 14.19. Found: C, 54.39; H, 5.63; N, 14.25.

2,7-Bis(chloromethyl)-5,10-dimethoxy-3,8-dimethylpyrimido[4,5-g]quinazoline-4,9(3*H*,8*H*)-dione (16). To a solution consisting of 15 mL of acetic acid and 0.5 mL of sulfuric acid was added 333 mg (0.766 mmol) of **12**. The resulting mixture was heated at 110 °C for 5.5 h. After the reaction mixture was cooled to room temperature, the solution was poured over ~50 mL of water and then buffered to pH 6 with sodium acetate. The resulting yellow precipitate was filtered, washed with water, and then dried. The dried solid was recrystallized from ethanol: 219-mg (72%) yield; mp 273–274 °C; TLC (ethyl acetate/methanol (9:1)), R_f = 0.34; IR (KBr) 1677, 1607, 1434, 1347, 1324, 1269, 1071, 1032, 795, 690 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 4.92 (4 H, s, methylenes), 3.95 (6 H, s, methoxys), 3.59 (6 H, s, 3- and 8-methyls); mass spectrum (EI) m/z 398 (M^+, $^{35}Cl^{35}Cl$), 400 (M^+, $^{37}Cl^{35}Cl$), 402 (M^+, $^{37}Cl^{37}Cl$). Anal. Calcd for $C_{16}H_{16}Cl_2N_4O_4$: C, 48.14; H, 4.04; N, 14.03. Found: C, 48.40; H, 4.03; N, 14.14.

2,7-Bis(bromomethyl)-5,10-dimethoxy-3,8-dimethylpyrimido[4,5-g]quinazoline-4,9(3*H*,8*H*)-dione (17). To a solution consisting of 15 mL of acetic acid and 0.5 mL of sulfuric acid was added 243 mg (0.463 mmol) of **13**. The resulting mixture was heated at 100 °C for 9.5 h. After the solution was allowed to cool to room temperature, the solvents were removed in vacuo to near dryness. The residue was triturated with water, and the resulting solids were filtered off. A second crop of solids was obtained by buffering the filtrate with sodium acetate to pH 6. The solids were combined, rinsed with water, and dried to afford the yellow-colored product: 156-mg (69%) yield; mp 263–264 °C; TLC (ethyl acetate/methanol (9:1)) R_f = 0.3; IR (KBr pellet) 1680, 1606, 1466, 1434, 1347, 1324, 1270, 1072, 1032, 690 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 4.92 (4 H, s, methylenes), 3.95 (6 H, s, methoxys), 3.59 (6 H, s, 3- and 8-methyls); mass spectrum (EI mode) m/z 486, 488 and 490 (M^+ for the ^{79}Br, ^{79}Br; ^{79}Br, ^{81}Br; ^{81}Br, ^{81}Br combinations, respectively, at an intensity ratio of 1:2:1).

2,7-Bis(bromomethyl)-5,10-dihydroxypyrimido[4,5-g]quinazoline-4,9(3*H*,8*H*)-dione (18). To a solution of 217 mg (0.447 mmol) of **14** in 50 mL of dry benzene, was added 3.2 mL (3.20 mmol) of 1 M BBr_3 in methylene chloride. The resulting mixture was refluxed for 5 h. Additional BBr_3 (1.3 mL, 1.3 mmol) was then added to the reaction mixture, and the mixture was refluxed for an additional 5 h. After the reaction mixture was

cooled to room temperature, methanol was added to destroy the excess BBr_3, and the solvents were removed in vacuo. The yellowish-red solid residue was redissolved in 5 mL of methanol and 75 mL of water added to the solution resulting in precipitation of a greenish-yellow solid. Filtration and washing the solids with water and then washing with diethyl ether gave pure **18**: 137-mg (71%) yield; >285 °C dec; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.42; IR (KBr pellet) 3328, 3024, 1669, 1649, 1419, 1271, 1245, 1225, 1038, 800 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 4.45 (4 H, s, methylenes); mass spectrum (EI solids probe) m/z 430, 432, and 434 (M^+ for the ^{79}Br, ^{79}Br; ^{79}Br, ^{81}Br; ^{81}Br, ^{81}Br combinations, respectively, at an intensity ratio of 1:2:1). Anal. Calcd for $C_{12}H_8Br_2N_4O_4 \cdot 0.5H_2O$: C, 32.68; H, 2.05; N, 12.70. Found: C, 32.84; H, 1.89; N, 12.10.

2,7-Bis(bromomethyl)pyrimido[4,5-*g*]quinazoline-4,5,9,10(3*H*,8*H*)-tetrone (19). To 105 mg (0.244 mmol) of **18** suspended in 15 mL of dry methanol was added 61 mg (0.268 mmol) of dichlorodicyanobenzoquinone (DDQ) and the reaction left to stir at room temperature for 1 h. The solids were filtered, washed with methanol, and dried to afford pure **19**: 95 mg (91%) yield; 274–275 °C dec; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.46; IR (KBr pellet) 3062, 3040, 2990, 2933, 1713, 1645, 1576, 1547, 1480, 1142 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 4.45 (4 H, s, methylenes); mass spectrum (EI, solids probe) m/z 428, 430, and 432 (M^+ for the ^{79}Br, ^{79}Br; ^{79}Br, ^{81}Br; ^{81}Br, ^{81}Br combinations, respectively, in an intensity ratio of 1:2:1). Anal. Calcd for $C_{12}H_6Br_2N_4O_4$: C, 33.52; H, 1.41; N, 13.03. Found: C, 33.89; H, 1.31; N, 12.50.

2,7-Bis(hydroxymethyl)-5,10-dihydroxy-3,8-dimethylpyrimido[4,5-*g*]quinazoline-4,9(3*H*,8*H*)-dione (20). To 172 mg (0.441 mmol) of **15**, suspended in 50 mL of dry benzene, was added an excess of 99% BBr_3 (up to 10-fold excess). The resulting mixture was refluxed for 7 h. After the reaction mixture was cooled to room temperature, methanol was added to quench the reaction. The solvent was evaporated off in vacuo and the product dried for several hours to remove hydrobromic acid. The product was then purified by rinsing successively with water and methanol: 106.5-mg (72%) yield; >245 °C dec; IR (KBr pellet) 3384, 1645, 1618, 1443, 1430, 1379, 1325, 1209, 1079, 965, 793 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 11.86 (2 H, s, 5- and 10-hydroxyls), 4.62 (4 H, s, methylenes), 3.61 (6 H, s, 3-, and 8-methyls); mass spectrum (EI mode) m/z 334 (M^+).

2,7-Bis(chloromethyl)-5,10-dihydroxy-3,8-dimethylpyrimido[4,5-*g*]quinazoline-4,9(3*H*,8*H*)-dione (21). To a solution of 105 mg (0.313 mmol) of **20** in 10 mL of dry dimethylformamide were added 66 mg (1.56 mmol) of LiCl and 191 mg (1.56 mmol) of (dimethylamino)pyridine. To this mixture was added 120 μL (1.56 mmol) of methanesulfonyl chloride and the resulting solution gently heated with a heat gun until it became homogeneous. After this mixture was stirred at room temperature for 6 h, it was combined with 30 mL of water and the resulting mixture allowed to stir for an additional 2 h. The resulting yellow precipitate was filtered, rinsed with water, and dried: 99 mg (85%) yield; mp >275 °C dec; IR (KBr pellet) 3036, 1645, 1603, 1421, 1372, 1343, 1270, 1240, 1015, 794 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 11.84 (2 H, s, 5- and 10-hydroxyl) 4.95 (4 H, s, methylenes), 3.65 (6 H, s, 3- and 8-methyls); mass spectrum (EI, solids probe) m/z 370 (M^+, $^{35}Cl^{35}Cl$), 372 (M^+, $^{37}Cl^{35}Cl$), 374 (M^+, $^{37}Cl^{37}Cl$). Anal. Calcd for $C_{14}H_{12}Cl_2N_4O_4$: C, 45.30, H, 3.26; N, 15.09. Found: C, 45.76; H, 3.47; N, 13.99.

2,7-Bis(chloromethyl)-3,8-dimethylpyrimido[4,5-*g*]quinazoline-4,5,9,10(3*H*,8*H*)-tetrone (22). To 32 mg (0.088 mmol) of **21**, suspended in 3 mL of 75% aqueous acetonitrile, was added 96 mg (0.175 mmol) of ceric ammonium nitrate (CAN). The starting material immediately went into solution, and after ~5-min reaction time, formation of a precipitate was observed. After the mixture was stirred for an additional 15 min, 3 mL of water was added to the reaction mixture and the resulting solution was extracted several times with chloroform. The extracts were dried (Na_2SO_4) and then were evaporated to a small volume. Addition of hexane resulted in crystallization of pure product: 16-mg (49%) yield; >275 °C dec; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.5; IR (KBr pellet) 3440, 1711, 1659, 1569, 1525, 1441, 1386, 1373, 1067, 978 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 4.97 (4 H, s, methylenes), 3.61 (6 H, s, 3- and 8-methyls); mass spectrum (EI, solids probe) m/z 370 (M^+ + 2, $^{35}Cl^{35}Cl$), 372 (M^+ + 2, $^{37}Cl^{35}Cl$), 374 (M^+ + 2, $^{37}Cl^{37}Cl$). Anal. Calcd for $C_{14}H_{10}Cl_2N_4O_4$: C, 45.55; H, 2.73; N, 15.18. Found: C, 45.30; H, 2.92; N, 14.27. The nitrogen percentage obtained experimentally deviates widely from the theoretical values. The ^1H NMR and mass spectra indicate that the material is pure and that the assigned structure correct, however.

2-Acetamido-3,6-dicarbamyl-1,4-dimethoxybenzene (23) was prepared by the two-step process described below. A mixture consisting of 1.74 g (6.47 mmol) of **3**, 200 mg of 5% Pd on carbon, and 200 mL of methanol were shaken under 50 psi of H_2 for 3 h. The crystallized product was separated from the catalyst by adding 200 mL of acetic acid, heating the mixture, and filtering through Celite. The solvents were evaporated in vacuo to afford a yellow oily residue, which upon addition of ethanol and sonication afforded the amine as a crude tan solid, 1.25-g (81%) yield. For further purification, the solid was dissolved in hot dimethylformamide and decolorized with activated charcoal. The solution was then filtered through Celite and the solvent evaporated off to give an oily residue. Addition of ethanol and then sonication resulted in crystallization of the pure amine as white flakes: mp 272–273 °C; TLC (chloroform/methanol (9:1)), R_f = 0.31; IR (KBr pellet) 3427, 3394, 3182, 1639, 1589, 1465, 1453, 1418, 1399, 1140 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 7.65, 7.56 and 7.48 (4 H, 3 br s, 3- and 6-carbamyls, no assignments made), 6.39 (1 H, s, 4-H), 6.27 (2 H, br s, amino), 3.78 and 3.64 (6 H, 2 s, 3- and 6-methoxys, no assignments made); mass spectrum (EI mode) m/z 239 (M^+). Anal. Calcd for $C_{10}H_{13}N_3O_4$: C, 50.21; H, 5.48; N, 17.56. Found: C, 49.95; H, 5.44; N, 17.21.

To a solution consisting of 20 mL acetic anhydride and 10 mL of acetic acid was added 225 mg (0.939 mmol) of the product obtained above. The mixture was heated at 40 °C for 4.5 h. After methanol was added to quench the reaction, the solvents were evaporated in vacuo and the residue was dissolved in hot dimethylformamide and then treated with activated charcoal. Filtration through Celite, evaporation of the solvent in vacuo, followed by addition of ethanol, and then sonication afforded the desired product as a white solid: 206-mg (78%) yield; mp 257–258 °C; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.52; IR (KBr pellet) 3172, 3167, 1697, 1658, 1517, 1456, 1411, 1383, 1253, 1112 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 9.38 (1 H, br s, acetamido NH), 7.72, 7.64, and 7.43 (4 H, 3 br s, 3- and 6-carbamyls, no assignments made), 7.24 (1 H, s, aromatic), 3.78 and 3.66 (6 H, 2 s, 1- and 4-methoxys, no assignments made), 1.97 (3 H, br s, acetamido methyl); mass spectrum (EI mode) m/z 281 (M^+).

7-(Methoxycarbonyl)-5,8-dimethoxy-2-methylquinazolin-4(3*H*)-one (24) was prepared by the following two-step procedure. A mixture consisting of 20 mL of ethanol, 10 mL of 10% aqueous sodium hydroxide, and 523 mg (1.86 mmol) of **23** was refluxed for 24 h. The solvent was evaporated off, and the resulting residue was redissolved in a minimum amount of water. To remove the salts by anion-exchange chromatography (Bio-Rad, AG1-X4, 200–400 mesh), the aqueous solution obtained above was made basic and placed on the column. After the column was washed with water, the product was eluted off with dilute aqueous hydrochloric acid. The eluant was evaporated to dryness in vacuo, and the solid residue was dissolved in hot ethanol. Addition of ethyl acetate afforded the ring-closed product as the carboxylic acid: 383-mg (78%) yield; TLC (2-propanol/water/ammonia (7:1:2)) R_f = 0.47; ^1H NMR (dimethyl-d_6 sulfoxide) δ 7.08 (1 H, s, 6-H), 3.87 (6 H, s, methoxys), 2.42 (3 H, s, 2-methyl); mass spectrum (EI mode) m/z 264 (M^+).

The carboxylic acid, 383 mg (1.45 mmol), 50 mL of methanol, and 2 mL of concentrated sulfuric acid were combined and refluxed for 10 h. The reaction mixture was evaporated in vacuo to an oil, which was poured over 50 mL of ice–water. The solution was neutralized with sodium carbonate and was then extracted with 3 × 75-mL portions of chloroform. The organic extracts were dried (Na_2SO_4) and were evaporated to a small volume. Addition of hexane afforded **24** as white flakes: 277-mg (69%) yield; mp 205–207 °C; TLC (2-propanol/water/ammonia (7:1:2)) R_f = 0.61; IR (KBr pellet) 1711, 1682, 1634, 1465, 1436, 1352, 1291, 1263, 1236, 1109 cm^{-1}; ^1H NMR (dimethyl-d_6 sulfoxide) δ 6.99 (1 H, s, 6-H), 3.89 and 3.83 (9 H, 2 s, methoxys, no assignments made), 2.33 (3 H, s, 2-methyl); mass spectrum (EI mode) m/z 278 (M^+). Anal. Calcd for $C_{13}H_{14}N_2O_5 \cdot 0.2H_2O$: C, 55.40; H, 5.15; N, 9.94. Found: C, 55.54; H, 4.86; N, 10.05.

7-(Methoxycarbonyl)-5,8-dimethoxy-2-methyl-6-nitroquinazolin-4(3*H*)-one (25). To 703 mg (2.53 mmol) of **24**, suspended in 25 mL of dry acetonitrile chilled to 10 °C, was added 434 mg (2.78 mmol) of NO_2BF_4. After the reaction mixture was stirred for 10 min at room temperature, the homogeneous solution was poured into 100 mL of ice–water. The resulting yellow solid was filtered, washed with water, and dried to afford pure product: 621-mg (76%) yield; mp 246–247 °C; TLC (ethyl acetate/ethanol (95:5)) R_f = 0.35; IR (KBr pellet) 2957, 1745, 1679, 1621, 1537, 1443, 1350, 1291, 1239 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 3.99, 3.91 and 3.87 (9 H, 3 s, methoxys, no assignments made), 2.42 (3 H, s, 2-methyl); mass spectrum (EI mode) m/z 323 (M^+). Anal. Calcd for $C_{13}H_{13}N_3O_7$: C, 48.30; H, 4.05; N, 13.00. Found: C, 48.32; H, 3.94; N, 12.82.

6-(Phenoxyacetamido)-7-(methoxycarbonyl)-5,8-dimethoxy-2-methylquinazolin-4(3*H*)-one (26) was prepared by the two-step procedure described below. A mixture consisting of 581 mg (1.80 mmol) of **25**, 50 mg 5% Pd/C, and 100 mL of methanol was shaken under 50 psi of H_2 for 4 h. The solution was filtered through Celite, and the solvents were evaporated off to afford the crude 6-amino derivative as an amber solid residue. The residue was recrystallized by dissolution in the minimum amount of hot ethanol followed by addition of hexane: yield 483 mg (92%); mp 209–210 °C; TLC (ethyl acetate/ethanol (95:5)) R_f = 0.25; IR (KBr pellet) 2944, 1716, 1670, 1641, 1459, 1434, 1333, 1308, 1231, 1046 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 5.25 (2 H, s, 6-amino), 3.88, 3.87 and 3.70 (9 H, 3 s, methoxys, no assignments made), 2.26 (3 H, s, 2-methyl); mass spectrum (EI mode) m/z 293 (M^+). Anal. Calcd for $C_{13}H_{15}N_3O_5 \cdot 0.1H_2O$: C, 52.92; H, 5.19; N, 14.24. Found: C, 52.73; H, 4.93; N, 14.01.

To 185 mg (0.630 mmol) of the amine obtained above, suspended in 20 mL of dry benzene, were added 100 μL (0.725 mmol) of phenoxyacetyl chloride and 60 μL (0.742 mmol) of pyridine. The resulting mixture was stirred at room temperature for 3.5 h. Evaporation of the solvent in vacuo to a solid residue, followed by addition of ~5 mL of aqueous ethanol and sonication, afforded 226 mg of crude product. The compound was then dissolved in chloroform and the solution decolorized with activated charcoal. After the charcoal was removed, the solvent was reduced to a small volume and the product crystallized by addition of hexane: 216-mg (80%) yield; mp 225–226 °C; TLC (ethyl acetate/methanol (9:1)) R_f = 0.32; IR (KBr pellet) 3389, 1726, 1629, 1627, 1514, 1497, 1456, 1239, 1226, 1062 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 12.25 (1 H, s, 3-NH), 9.73 (1 H, s, acetamido NH), 7.37–7.31 and 7.01–6.98 (5 H, complex multiplets, aromatic), 4.69 (2 H, s, methylene), 3.93, 3.74 and 3.67 (9 H, 3 s, methoxys, no assignments made), 2.36 (3 H, s, 2-methyl); mass spectrum (EI mode) m/z 427 (M^+). Anal. Calcd for $C_{21}H_{21}N_3O_7$: C, 59.01; H, 4.95; N, 9.83. Found: C, 59.04; H, 4.84; N, 9.67.

2-(Phenoxyacetamido)-5,10-dimethoxy-7-methylpyrimido[4,5-g]quinazoline-4,9(3*H*,8*H*)-dione (27). A mixture of 309 mg (0.723 mmol) of **26**, 3.5 mg (0.072 mmol) of sodium cyanide,[26] and 50 mL of saturated methanolic ammonia was heated in a steel bomb at 80 °C for 24 h. The solvent was then evaporated in vacuo, and the residue was dissolved in a small volume of water. The product crystallized from solution upon adjusting the pH to 6 with concentrated hydrochloric acid. The yellow product was recrystallized from aqueous dimethylformamide: 177-mg (62%) yield; 312–314 °C dec; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.6; IR (KBr pellet) 2971, 2933, 2874, 1687, 1627, 1600, 1498, 1279, 1211, 1072 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 7.32 (2 H, t, J = 7.6 Hz, aromatic, no assignments made) 7.09 (2 H, d, J = 8.8 Hz, aromatic, no assignments made), 6.98 (1 H, t, J = 7.1 Hz, aromatic, no assignments made), 5.01 (2 H, s, methylene), 3.88 and 3.81 (6 H, 2 s, 5- and 10-methoxys, no assignments made), 2.35 (3 H, s, 7-methyl); mass spectrum (EI mode) m/z 394 (M^+). Anal. Calcd for $C_{20}H_{18}N_4O_5 \cdot 0.75H_2O$: 58.89; H, 4.81; N, 13.73. Found: C, 58.64; H, 4.66; N, 13.67.

2-(Chloromethyl)-5,10-dihydroxy-7-methylpyrimido[4,5-g]quinazoline-4,9(3*H*,8*H*)-dione (28) was prepared by the two-step synthesis described below. To a suspension consisting of 191 mg (0.483 mmol) of **27** in 30 mL of dry benzene was added 3.8 mL (3.80 mmol) of 1 M BBr_3. The resulting mixture was refluxed for 24 h. After the solution was cooled to room temperature, methanol was added to quench the reaction. The solvents were removed in vacuo, and the precipitated residue was combined with ~20 mL of water. The resulting solid (the bromomethyl derivative of **28**) was filtered, rinsed with water, and then rinsed with diethyl ether: 105-mg (62%) yield; mp >250 °C dec; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.17; IR (KBr) 3052, 3041, 3021, 1654, 1619, 1416, 1376, 1273, 1226, 1039 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 12.74 (2 H, br s, 5- and 10-OH), 11.88 and 11.67 (2 H, 2 br s, 3- and 8-NH, no assignments made), 4.44 (2 H, s, methylene), 2.39 (3 H, d, J = 3.7 Hz, 7-methyl coupled to 8-NH); mass spectrum (EI mode) m/z 354 (M^+, ^{79}Br), 356 (M^+, ^{81}Br).

In 5 mL of dry dimethylformamide, 103 mg (0.291 mmol) of the bromomethyl derivative and 123 mg (2.91 mmol) of lithium chloride were combined, and the resulting mixture was stirred at room temperature for 2.5 h. The solvent was evaporated in vacuo to an oily residue with minimal heating. The addition of ~20 mL of water precipitated the product, which was filtered and then was washed with water: 82-mg (91%) yield; mp >290 °C dec; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.25; IR (KBr) 3070, 3062, 1657, 1621, 1491, 1418, 1377, 1272, 1225, 1040 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 11.88 and 11.77 (2 H, 2 br s, no assignments made), 4.57 (2 H, s methylene), 2.39 (3 H, s, 2-methyl); mass spectrum (EI mode, solids probe) m/z 274 (M^+ – Cl).

1,4-Bis(methylcarbamyl)-2,5-dimethoxy-6-nitrobenzene (29). To 75 mL of a 10% methanolic methylamine solution was added 1.15 g (3.84 mmol) of **4**. After the reaction mixture was stirred at room temperature for 5 h, the solvent was evaporated to dryness and the oily residue was dissolved in a minimum amount of hot ethanol. Addition of hexane to this solution afforded **29** as an orange-colored solid: 1.03-g (91%) yield; mp 233–235 °C; TLC (chloroform/ethanol (95:5)) R_f = 0.25; IR (KBr pellet) 3323, 1661, 1649, 1563, 1543, 1484, 1403, 1379, 1231, 1061 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 8.48 (2 H, 2 overlapping quartets, 1- and 4-carbamyl protons), 7.38 (1 H, s, 3-H aromatic), 3.86 and 3.74 (6 H, 2 s, 2- and 5-methoxys, no assignments made), 2.80 and 2.70 (6 H, 2 d, J = 2.4 Hz, 1- and 4-carbamyl methyls, no assignments made); mass spectrum (EI mode) m/z 297 (M^+).

2-(Chloroacetamido)-1,4-bis(methylcarbamyl)-3,6-dimethoxybenzene (30) was prepared by the two-step procedure described below. A mixture consisting of 1.04 g (3.49 mmol) of **29**, 200 mg of 5% Pd/C, and 200 mL of methanol was shaken overnight under 50 psi of H_2. The Pd/C was removed by filtering the reaction mixture through a Celite pad using methanol as the eluant. The filtrate was evaporated to an oily residue, which was dissolved in 20 mL of hot ethanol. Addition of hexane afforded a pinkish-colored solid, which was rinsed with cold water: 700-mg (75%) yield; TLC (chloroform/methanol (9:1)) R_f = 0.56; IR (KBr pellet) 3337, 3321, 1644, 1631, 1589, 1550, 1461, 1418, 1358, 1224 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 8.14 and 8.09 (2 H, 2 br q, J = 2.7 Hz, 1- and 4-carbamyls protons), 6.33 (1 H, s, 5-H aromatic), 5.93 (2 H, d, J = 2.8 Hz, 2-amino), 3.75 and 3.61 (6 H, 2 s, 3- and 6-methoxys, no assignments made), 2.78 and 2.75 (6 H, 2 d, J = 2.3 Hz, 1- and 4-carbamylmethyls, no assignments made); mass spectrum (EI mode) m/z 267 (M^+).

In 100 mL of dry dimethylformamide, 3.0 g (11.2 mmol) of the product obtained above, 4.45 mL (55 mmol) dry pyridine, and 1.31 mL (17.0 mmol) of chloroacetyl chloride were combined and stirred at room temperature for 2 h. The solvent was then evaporated in vacuo without heating to an oily residue, which was combined with ~100 mL of water and sodium bicarbonate such that the pH ~7. The solvent was then evaporated in vacuo to a dry residue, which was extracted several times with chloroform. The chloroform portions were combined, dried (Na_2SO_4), and evaporated to dryness to afford a tan solid: 3.4 g (90%) of crude **30**. For pure material, the following procedure was carried out. After 163 mg of compound **30** was dissolved in the minimum amount of chloroform and the solution allowed to sit for 1 h, the oily residue was filtered off and hexane was added until the solution became cloudy. After sitting for 24 h, the white solids were filtered and dried to give 144 mg of **30**: mp 228–229 °C; TLC (ethyl acetate/methanol (9:1)) R_f = 0.21; IR (KBr pellet) 3275,

(26) The cyanide is used as a catalyst for the ester to amide transformation; see: Högberg, T.; Ström, P.; Ebner, M.; Rämsby, S. *J. Org. Chem.* **1987**, *52*, 2033.

1667, 1641, 1564, 1525, 1477, 1413, 1290, 1240, 1079 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 9.69 (1 H, s, acetamido NH), 8.24 (1 H, br q, J = 2.4 Hz, carbamyl methyl, no assignment made), 7.83 (1 H, br q, J = 2.4 Hz, carbamyl methyl, no assignment made), 7.19 (1 H, s, 5-H aromatic) 4.20 (2 H, s, methylene), 3.77 and 3.63 (6 H, 2 s, 3- and 6-methoxys, no assignments made), 2.80 and 2.67 (6 H, 2 d, J = 2.3 Hz, 1- and 4-carbamyl methyls, no assignments made); mass spectrum (EI mode) m/z 343 (M$^+$, ^{35}Cl), 345 (M$^+$, ^{37}Cl).

2-(Chloroacetamido)-1,4-bis(methylcarbamyl)-3,6-dimethoxy-5-nitrobenzene (31). To 50 mL of red fuming nitric acid, chilled to 0 °C, was added 2.3 g (6.69 mmol) of **30**. After the mixture was stirred at 0 °C for 45 min, 100 mL of ice water was added and the mixture quickly extracted with ethyl acetate. The ethyl acetate extract was dried over Na_2SO_4, evaporated down to a small volume, and placed on a silica gel column. Gradient chromatography (ethyl acetate to 5% methanolic ethyl acetate) afforded the desired produce as a pure white solid: 1.5-g (58%) yield; TLC (ethyl acetate/methanol (9:1)), R_f = 0.40; mp 259–260 °C; IR (KBr pellet) 3270, 1676, 1651, 1568, 1534, 1472, 1404, 1373, 1315, 1054 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 9.99 (1 H, s, acetamido NH), 8.64 and 8.28 (2 H, 2 s, J = 2.3 Hz, 1- and 4-carbamyl protons, no assignments made), 4.25 (2 H, s, methylene), 3.79 and 3.68 (6 H, 2 s, 3- and 6-methoxys, no assignments made), 2.73 (6 H, 2 overlapping d, J = 4.4 Hz, 1- and 4-carbamyl methyls, no assignments made); mass spectrum (EI mode) m/z 388 (M$^+$, ^{35}Cl), 390 (M$^+$, ^{37}Cl).

2-(Chloroacetamido)-1,4-bis(methylcarbamyl)-5-amino-3,6-dimethoxybenzene (32). A mixture consisting of 930 mg (2.39 mmol) of **31**, 100 mg of 5% Pd/C, and 200 mL of methanol was shaken under 50 psi of H_2 for 2 h. The spent Pd/C was removed by filtration through a Celite pad using methanol as eluant. The solvent was evaporated off and the solid residue redissolved in chloroform. Flash chromatography using ethyl acetate as eluant gave the desired product as a white solid: 749-mg (87%) yield; mp 242–244 °C; TLC (ethyl acetate/methanol (9:1)) R_f = 0.29; IR (KBr pellet) 3275, 1698, 1682, 1629, 1578, 1536, 1456, 1426, 1407, 1329 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 9.34 (1 H, s, acetamido NH), 8.13 and 7.69 (2 H, 2 br q, J = 2.3 Hz, 1- and 4-carbamyl protons, no assignments made), 5.64 (2 H, s, 5-amino), 4.16 (2 H, s, methylene), 3.63 and 3.57 (6 H, 2 s, 3- and 6-methoxys, no assignments made), 2.77 and 2.69 (6 H, 2 d, J = 2.3 Hz, 1- and 4-carbamyl methyls, no assignments made); mass spectrum (EI mode) m/z 358 (M$^+$, ^{35}Cl), 360 (M$^+$, ^{37}Cl).

2-(Chloromethyl)-5,10-dimethoxy-3,7,8-trimethylpyrimido[4,5-*g*]quinazoline-4,9(3*H*,8*H*)-dione (33) was synthesized by the following two-step procedure. In 50 mL of dry dimethylformamide were added 663 mg (1.85 mmol) of **32**, 164 μL (2.03 mmol) of dry pyridine, and 145 μL (2.03 mmol) of acetyl chloride. After the mixture was stirred at room temperature for 0.5 h, the solvent was removed in vacuo and the solid residue was dried for 1 h: TLC (*n*-butanol/acetic acid/water (5:2:3)), R_f = 0.5; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 9.69 (1 H, s, acetamido NH, no assignment made), 9.35 (1 H, br s, acetamido NH, no assignment made), 7.82 and 7.72 (2 H, 2 br q, carbamyl protons, no assignments made), 4.21 (2 H, s, methylene), 3.65 and 3.64 (6 H, 2 s, 5- and 10-methoxys, no assignments made), 2.70 (6 H, d, J = 3.9 Hz, carbamyl methyls), 1.98 (3 H, br s, acetyl); mass spectrum (EI mode) m/z 400 (M$^+$, ^{35}Cl), 402 (M$^+$, ^{37}Cl).

To the dried residue obtained above were added 30 mL of acetic acid and 3 mL of concentrated sulfuric acid. The resulting solution was heated at 100 °C for 4 h. The solution was then evaporated in vacuo to an oily residue, to which was added 50 mL of water. After the solution was neutralized to ~pH 7 with sodium bicarbonate, the solution was extracted several times with ethyl acetate (until extracts contained no product). The ethyl acetate extracts were dried over Na_2SO_4 and evaporated to a residue. Flash chromatography of the residue on silica gel using ethyl acetate as eluant afforded pure product. Recrystallization was carried out from ethanol: overall yield 365 mg (54%); mp 214–216 °C; TLC (ethyl acetate/methanol (8:2)) R_f = 0.40; IR (KBr) 1687, 1678, 1605, 1466, 1422, 1334, 1322, 1261, 1067, 1030 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 4.90 (2 H, s, methylene), 3.93 and 3.92 (6 H, 2 s, 5- and 10-methoxys, no assignments made), 3.58 and 3.50 (6 H, 2 s, N (3- and 8-) methyls, no assignments made), 2.60 (3 H, s, 7-methyl); mass spectrum (EI mode) m/z 364 (M$^+$, ^{35}Cl), 366 (M$^+$, ^{37}Cl). Anal. Calcd for $C_{16}H_{17}ClN_4O_4$: C, 52.68; H, 4.69; N, 15.35. Found: C, 52.49; H, 4.59; N, 15.64.

2-(Chloromethyl)-3,7,8-trimethylpyrimido[4,5-*g*]quinazoline-4,5,9,10(3*H*,8*H*)-tetrone (34). To 144 mg (0.395 mmol) of **33**, dissolved in 50 mL of acetonitrile, was added 650 mg (1.19 mmol) of ceric ammonium nitrate in 15 mL of water. After the solution was stirred at room temperature for 2 h, the solvents were evaporated in vacuo and the residue was combined with ~20 mL of water. The aqueous solution was extracted with chloroform until product was no longer observed in the extracts. Pure product was obtained by drying (Na_2SO_4) and evaporating off the chloroform and then prompting crystallization by addition of ethanol to the residue: 365-mg (54%) yield; mp >360 °C dec; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.34; IR (KBr) 1710, 1656, 1563, 1522, 1431, 1385, 1362, 1306, 1068, 976 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 4.97 (2 H, s, methylene), 3.60 (3 H, s, 3-methyl), 3.53 (3 H, s, 8-methyl), 2.68 (3 H, s, 7-methyl); mass spectrum (EI mode, solids probe) m/z 336 (M$^+$ + 2, ^{35}Cl), 338 (M$^+$ + 2, ^{37}Cl). Anal. Calcd for $C_{14}H_{11}ClN_4O_4$: C, 50.23; H, 3.31; N, 16.74. Found: C, 50.02; H, 3.20; N, 16.67.

2-(Chloromethyl)-5,10-dihydroxy-3,7,8-trimethylpyrimido[4,6-*g*]quinazoline-4,8(3*H*,8*H*)-dione (35). To a solution consisting of 12.5 mg (0.037 mmol) of **34** in 20 mL of chloroform was added 20 mL of aqueous sodium dithionite. The resulting mixture was shaken three times in a separatory funnel. The chloroform layer was removed, washed with water, and then dried over Na_2SO_4. The extract was evaporated in vacuo to ~2 mL, which upon addition of hexane afforded the desired product as a bright-yellow solid: 9.6-mg (77%) yield; (77%); mp >265 °C dec; TLC (*n*-butanol/acetic acid/water (5:2:3)) R_f = 0.22; IR (KBr) 1642, 1601, 1454, 1423, 1385, 1373, 1336, 1235, 1010, 796 cm^{-1}; ^{1}H NMR (dimethyl-d_6 sulfoxide) δ 12.00 and 11.73 (2 H, 2 s, 5- and 10-OHs, no assignments made), 4.93 (2 H, s, methylene), 3.64 and 3.55 (6 H, 2 s, 3- and 8-methyls, no assignments made), 2.61 (3 H, s, 7-methyl); mass spectrum (EI mode, solids probe) m/z 336 (M$^+$, ^{35}Cl), 338 (M$^+$, ^{37}Cl).

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