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Design of a Cyclopropyl Quinone Methide Reductive Alkylating Agent. 2

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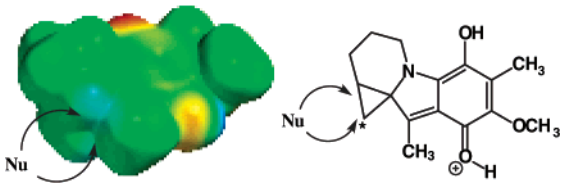

A cyclopropyl quinone methide is formed by elimination of a leaving group from an appropriately functionalized hydroquinone. The presence of a carbon spacer results in the formation of a fused ring rather than the classic methide species. Discussed herein is cyclopropyl quinone methide formation from a pyrido[1,2-*a*]indole ring system. Both nucleophilic and electrophilic attack on the fused cyclopropane ring results in pyrido[1,2-*a*]indole and azepino[1,2-*a*]indole products. The stereoelectronic effect plays less a role in the relatively flexible pyrido[1,2-a]indole system compared to its role in the pyrrolo[1,2-a]-indole system. A ^{13}C label on the fused cyclopropane ring permitted the rapid identification of complex rearrangement products observed in this study.

Introduction

Quinone methide chemistry is of general interest since many naturally occurring and synthetic quinones can form this reactive species upon two-electron reduction and leaving group elimination.[1−9] Previously, we designed a system that could form a cyclopropyl quinone methide from a pyrrolo[1,2-*a*]-fused system upon reduction of the quinone.[10] The inspiration for the cyclopropyl quinone methide design came from the mitosenes[11−13] and the A-ring of CC-1065.[14] While the mitosene hydroquinone affords the quinone methide by elimination of a leaving group, the present hydroquinone systems can eliminate the leaving group only by formation of a fused cyclopropane ring.

Presented herein are the synthesis of **1**, and the study of the fate of its cyclopropyl quinone methide **2**, Scheme 1. A ^{13}C label at the starred center of **1** permitted the rapid identification of products resulting from the nucleophile-mediated opening of the cyclopropane ring to afford ring retention and ring expansion products, **3** and **4**, respectively, in the inset of Scheme 1. This study represents a continuation of our use of ^{13}C labeling to elucidate methide chemistry.[10,15,16] The stereoelectronic effect, along with steric effects, controls the nucleophilic attack on the fused cyclopropane ring resulting in different ring opening paths for pyrido[1,2-*a*]-fused cyclopropyl quinone methide (**2**) compared to the previously reported pyrrolo[1,2-*a*]-fused analogue **5**,[10] Scheme 2. The more flexible pyrido[1,2-*a*] ring of **2** permits both nucleophilic attacks shown in the inset of Scheme 1 resulting in formation of both pyrido[1,2-*a*]indole **3** and azepino-[1,2-*a*]indole **4**, with the latter favored by large nucleophiles. In contrast, the pyrrolo[1,2-*a*]-fused system **5** affords only **6**

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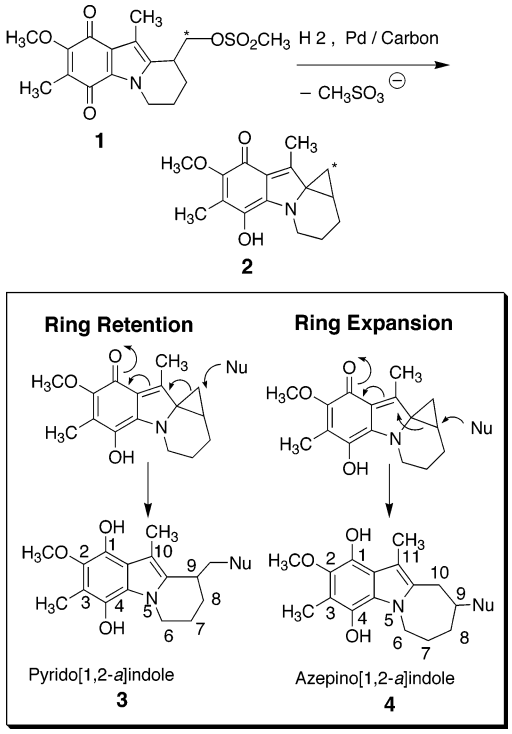
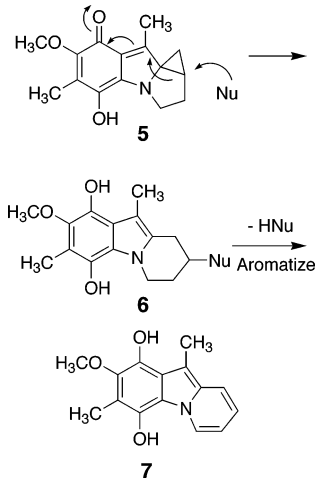
SCHEME 1



SCHEME 2

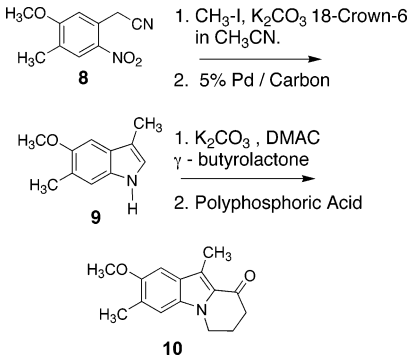


upon nucleophilic attack regardless of the size of the nucleophile.[10] The pyrido-fused product **6** eliminates the nucleophile to afford, upon air oxidation, the aromatized product **7**. In contrast, the nucleophile trapping products of cyclopropyl quinone methide **2** do not eliminate readily because products are not aromatic.

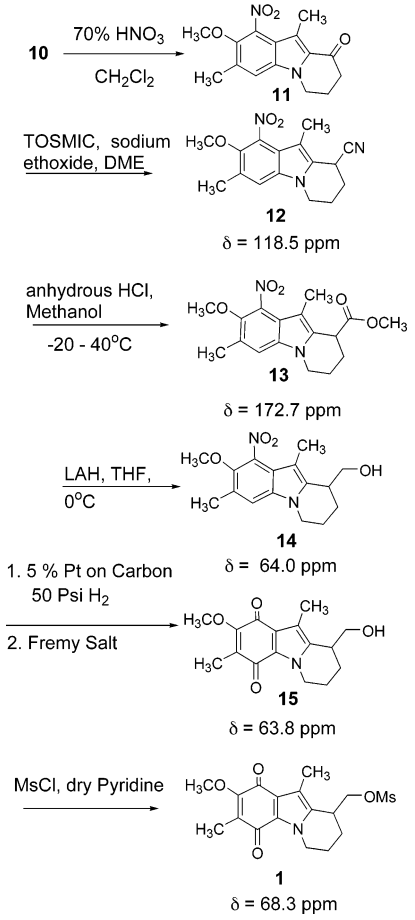
Results and Discussion

Synthesis. The synthesis of **1** was accomplished in 10 steps from commercially available 2-methyl-4-nitroanisole as outlined in Schemes 3 and 4. Introduction of the acetonitrile moiety into this starting material was carried out through "vicarious nucleophilic substitution" to afford **8**.[17,18] The acetonitrile moiety

SCHEME 3



SCHEME 4



was then methylated, followed by reductive cyclization to the indole **9**. The presence of the 3-methyl group prevented substitution reactions at this position of the indole ring during the synthesis of **1**. The annulation of the pyrido ring to afford the pyrido[1,2-*a*]indole **10** was accomplished by condensation of **9** with γ-butyrolactone followed by dehydrative cyclization using hot polyphosphoric acid. This annulation procedure has been employed for the preparation of other pyrido[1,2-*a*]indole analogues.[19]

Nitration of **10** to afford **11** was carried out in dichloromethane with 67% nitric acid. Replacement of the ketone group of **11** with a cyano group to afford **12** was carried out by

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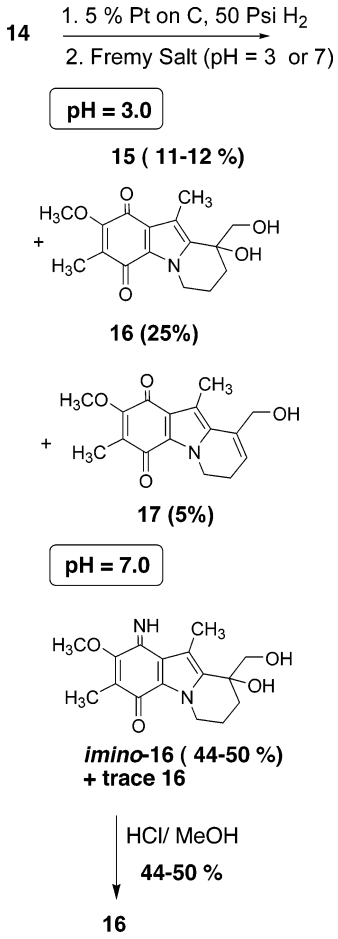
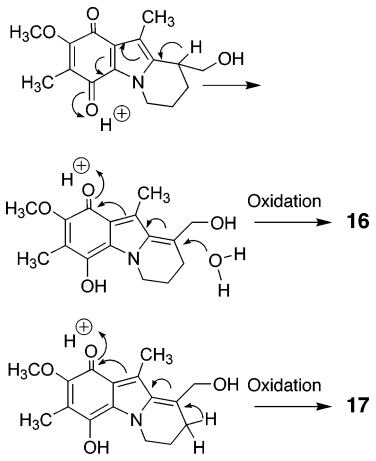
SCHEME 5



treatment with tosylmethyl isocyanide anion.[20,21] The resulting nitrile **12** was then exchanged with Na^{13}CN in DMSO at 80 °C to afford the ^{13}C enriched nitrile analogue **12*** in a 50% yield. Dry HCl was added to pyrido[1,2-*a*]indole nitrile **12** in methanol solution resulting in formation of the imino ester, which was treated with 3N HCl for 24 h to afford **13**. The conversion of **13** to hydroxymethyl pyrido[1,2-*a*]indole **14** as illustrated in Scheme 4 was uneventful. However, the conversion of **14** to **15** by catalytic reduction/Fremy oxidation at pH = 3 occurred in low yield with noteworthy side reactions discussed below. Finally, the hydroxymethyl indolequinone **15** was treated with methanesulfonyl chloride/pyridine to give methanesulfonoxymethyl indolequione **1**.

Fremy oxidation of reduced **14** (nitro to amino) afforded a variety of products depending on the pH of the reaction, Scheme 5. All reaction mixtures afford varying quantities of the diol **16**, a reaction not seen with the pyrrolo[1,2-*a*] fused analogues.[10] It is proposed that indole quinone **15** readily tautomerizes to the quinone methide species that either traps water or loses a proton to afford **16** and **17**, respectively (Scheme 6). The ease of tautomerization of the pyrido[1,2-*a*] fused analogues is attributed to less strain of the methide when present in a six-membered ring. The high yield preparation of **16** was carried out by Fremy oxidation to the iminoquinone at pH 7[22] to afford imino-**16** followed by acid-catalyzed imine hydrolysis.

SCHEME 6



Cyclopropyl Quinone Methide Chemistry. The product studies described below provided indirect evidence for the formation of the cyclopropyl quinone methide species **2** shown in Scheme 1. Product studies revealed that **2** has three possible fates: proton (electrophile) trapping resulting in alkane formation, nucleophile trapping, and dimerization (which is a combination of electrophile and nucleophile trapping). Some of these products are converted to other products by tautomerization processes. Unlike the cyclopropyl quinone methide **5**,[10] shown in Scheme 2, the stereoelectronic effect predicts no preferred path for cyclopropane ring opening because of ring flexibility.

Reduction of **1** and incubation in methanol followed by aerobic workup afforded a complex mixture of products that were initially analyzed by ^{13}C NMR, Figure 1. This spectrum clearly shows the formation of four types of products based on the chemical shifts of the label: oxygen-substituted ^{13}C (74 ppm), allylic or benzylic ^{13}C (45 ppm), ^{13}C with oxygen on a neighboring carbon (29 ppm), and alkyl ^{13}C (21 ppm). Isolation and identification studies confirmed the presence of each of these types of products, Scheme 7. Nucleophilic attack on the cyclopropyl quinone methide **2** affords pyrido[1,2-*a*]indole **21** and azepino[1,2-*a*]indole **22** upon oxidative workup as illustrated in Scheme 7. Initially, nucleophilic attack on the cyclopropane ring of **2** affords the hydroquinone derivatives that are oxidized to the quinones upon aerobic workup. Inspection of the minimized molecular model of **2** in Figure 2 reveals that pyrido-[1,2-*a*]indole and azepino[1,2-*a*]indole formation upon nucleophilic attack are both favored by the stereoelectronic effect. Ideally, the breaking bond is 45° to 90° to the π system so that there is maximal overlap with the developing p orbital. For example, nucleophilic attack of the CC-1065 A ring at the least substituted carbon of the cyclopropane ring, and subsequent bond breaking, results in good overlap (45° out of the π plane) of the developing p orbital with the π system.[23] In contrast, attack at the more substituted carbon would result in p orbital development orthogonal to the π system. Similarly, the stereoelectronic effect correctly predicted that cyclopropyl quinone **5** would undergo nucleophilic attack only at the more substituted carbon.[10] In contrast to **5** and the CC-1065 A ring, both nucleophilic attack paths on **2** will result in good overlap between the developing p orbital and the π system. The greater flexibility of the tetrahydropyrido ring of **2**, compared to that

(20) Oldenziel, O. H.; van Leusen, A. M. *Synth. Commun.* **1972**, *2* (5), 281−283.
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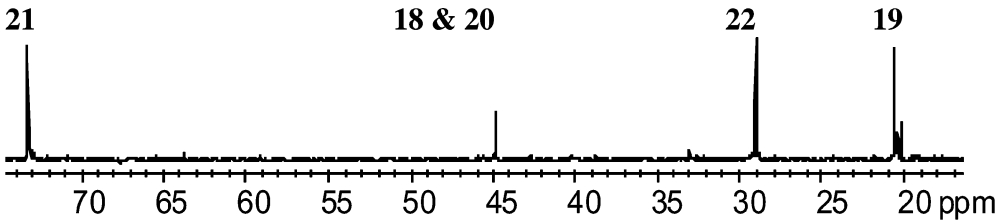
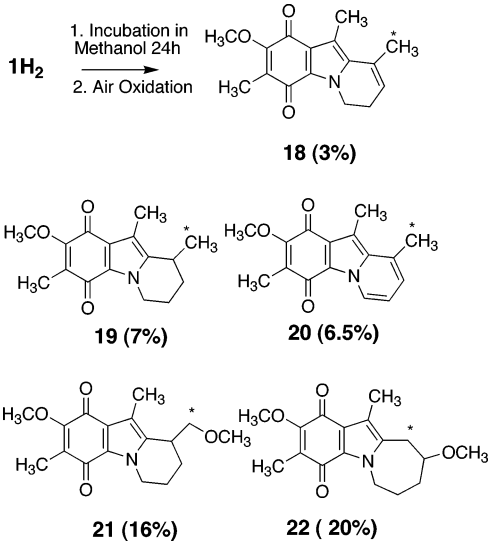


FIGURE 1. Enriched ^{13}C NMR of the methanolic solvolysis products of reduced **1**.

SCHEME 7



of the pyrrolo ring of **5**, accounts for the lack of regioselectivity in nucleophilic attack.

The nucleophile trapping products **21** and **22** are stable because these systems cannot aromatize. In both products the leaving group (formally the nucleophile) is either exocyclic or part of a seven-membered ring. In contrast, nucleophile trapping by the cyclopropyl quinone methide **5** affords a product that readily aromatizes to the fused pyrido system, **5** → **6** → **7** in Scheme 2. Therefore adducts of **2** with biological nucleophiles were found to be stable (see next section).

In addition to nucleophile trapping, the cyclopropyl quinone methide **2** traps a proton to afford **19** that is then converted to

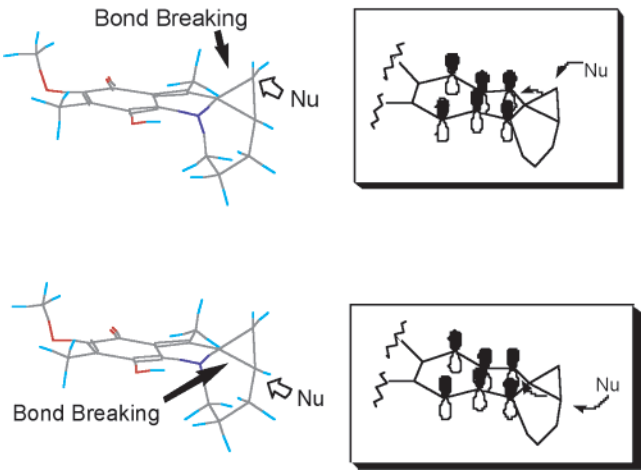
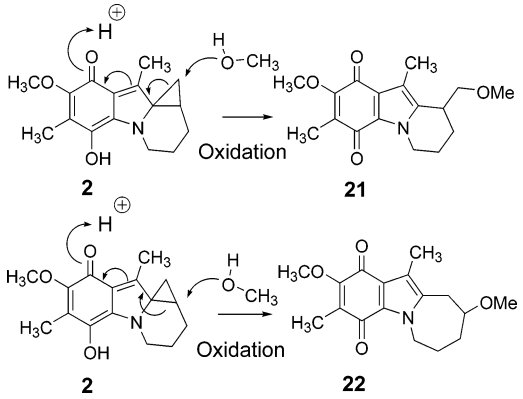
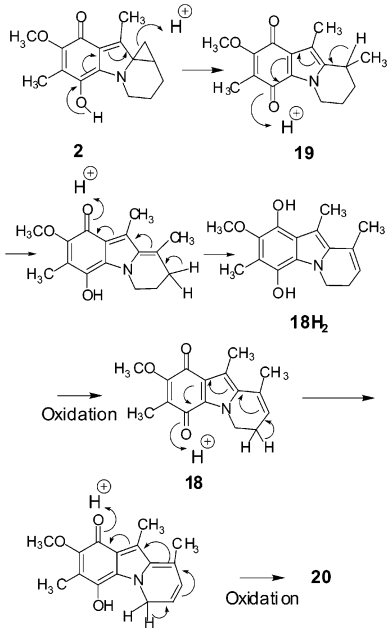


FIGURE 2. Minimized molecular models of the pyrido[1,2-*a*]indole cyclopropyl quinone methide reveal the absence of a stereoelectronic effect on cyclopropane ring opening.

SCHEME 8



SCHEME 9



18 and **20** by a series of tautomerizations and oxidations, Scheme 8. The process that converts **19** to reduced **18** (**18H$_2$**) is essentially an internal redox reaction wherein electrons flow from the fused tetrahydropyrido ring to the quinone ring by two tautomerizations. Aerobic oxidation of **18H$_2$** followed by two tautomerizations and aerobic oxidation again affords the aromatized fused pyrido ring of **20**, Scheme 9.

Hydrolysis studies of reduced **1** in pH 7.4 tris buffer afforded a mixture that was initially analyzed by ^{13}C NMR, Figure 3. The spectrum reveals the presence of trace starting material **1** and three products identified as **15**, **23**, and **24**, Scheme 10. The formation of **15** and **23** provide evidence of nucleophile attack paths illustrated in Figure 2. The dimer **24** must have resulted from the reaction between two molecules of **2** with

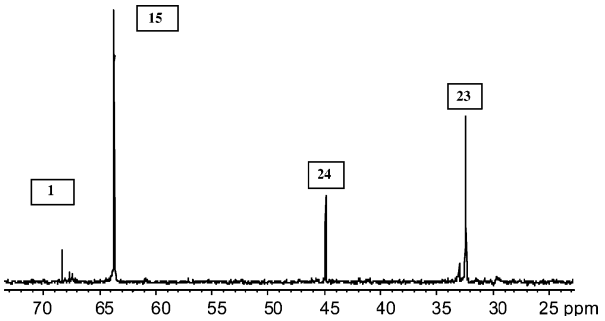
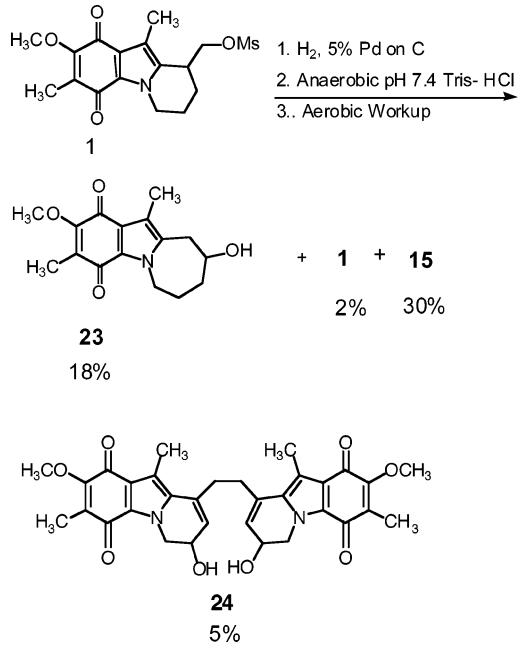


FIGURE 3. Enriched ^{13}C NMR of the hydrolysis products of reduced **1** in pH 7.4 tris buffer.

SCHEME 10



one reactant acting as a nucleophile and the other acting as electrophile. A series of reactions after the coupling, including tautomerizations, water trapping, water elimination, and oxidations eventually affords the final product **24** as illustrated in Scheme 11. The presence of a highly extended quinone methide that traps water by a 1,8-addition reaction (inset of Scheme 11) accounts for the allylic alcohol of **24**.

Cyclopropyl Quinone Methide Trapping of 5′−dGMP. To assess the trapping of biological nucleophiles, **2** was generated in the presence of 5′−dGMP. Reductive alkylation of 5′−dGMP at pH 7.4 afforded a mixture of phosphate adducts **25** and **26** that could not be separated by reverse phase chromatography, Scheme 12. The ^{13}C NMR spectrum of the purified mixture shown in Figure 4 reveals that the pyrido[1,2-*a*]indole **25** was the major product with trace amounts of azepino[1,2-*a*]indole **26** present. Since the stereoelectronic effect favors either product, steric effects must dictate nucleophilic attack at the least hindered cyclopropane carbon of **2** to afford **25**. In contrast, the nucleophile trapping reactions of cyclopropyl quinone methide **5** with 5′−dGMP and DNA are controlled exclusively by the stereoelectronic effect.[10]

Conclusions

Cyclopropyl quinone methide **2** formation from the pyrido[1,2-*a*]indoledione ring system **1** has been documented employing ^{13}C NMR and product isolations. Both nucleophilic and electrophilic attack on the fused cyclopropane ring of **2** results in pyrido[1,2-*a*]indole (ring retention) and azepino[1,2-*a*]indole (ring expansion) products. The stereoelectronic effect plays no discernible role in nucleophilic attack on the flexible pyrido[1,2-*a*]indole system of cyclopropyl quinone methide **2**. In contrast, the stereoelectronic effect dictates nucleophilic attack in the more rigid pyrrolo[1,2-*a*]indole system of cyclopropyl quinone methide **5**.[10] However, steric effects resulting in nucleophilic attack occurs at the least hindered cyclopropane carbon of **2** in the case of a large nucleophile. Nucleophile trapping products of **2** are stable and do not undergo aromatization, as was the case with nucleophile trapping products of **5**. The latter products readily eliminate the nucleophile, and then air oxidizes to afford a stable aromatic ring.[10] Thus the cyclopropyl quinone methide **2** appears to be a stable alkylation product suitable for biological systems. Another conclusion is that the 9-position of the pyrido[1,2-*a*]indoledione ring system is susceptible to tautomerization even under mild conditions. The tautomerization of the C(9) proton to afford a quinone methide is favored by the six-membered pyrido ring. Water addition to the quinone methide followed by air oxidation of the resulting hydroquinone affords the 9-hydroxy derivative. Similar tautomerization/oxidation reactions have been noted in other pyrido[1,2-*a*]indoledione derivatives[24] The pyrrolo[1,2-*a*]indoledione does not readily undergo tautomerization/oxidation reactions because of the strain of the methide in the five-membered pyrrolo ring. Currently, we are using the C(9) oxidation reaction to design novel dual alkylating agents based on the pyrido[1,2-*a*]indoledione ring system.

Experimental Section

All analytically pure compounds were dried under high vacuum in a drying pistol heated with refluxing methanol. Fremy salt was purchased from a commercial supplier, stored over calcium chloride desiccant in a refrigerator, and used within a month. Some compounds contained fractional amounts of water of crystallization that were determined from the elemental analyses found. Some trace byproducts were identified on the basis of spectral data without elemental analyses. Uncorrected melting points 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 and ^{13}C NMR spectra were obtained on a 300 MHz spectrometer, and chemical shifts are reported relative to TMS. The NMR peak assignments of some compounds were assigned on the basis of two-dimensional experiments and ^{13}C labeling, and these compounds have peak assignments.

3,6-Dimethyl-5-methoxyindole (9). Synthesis was carried out in two steps from the reported compound **8**[25] as follows: To a solution of 5.0 g (24.2 mmol) of **8** and 20 g (144.7 mmol) of K_2CO_3 in 100 mL of dry acetonitrile was added 100 mg of 18-crown-6 and 2.0 mL of CH_3I. The mixture was heated to reflux and stirred under nitrogen for 15 h. After completion of the reaction (monitored by TLC), the mixture was cooled to room temperature, the salt was filtered off, and the solvent evaporated to a residue under vacuum. This residue was dissolved into 150 mL of dichloromethane and filtered again to remove salts. The solvent

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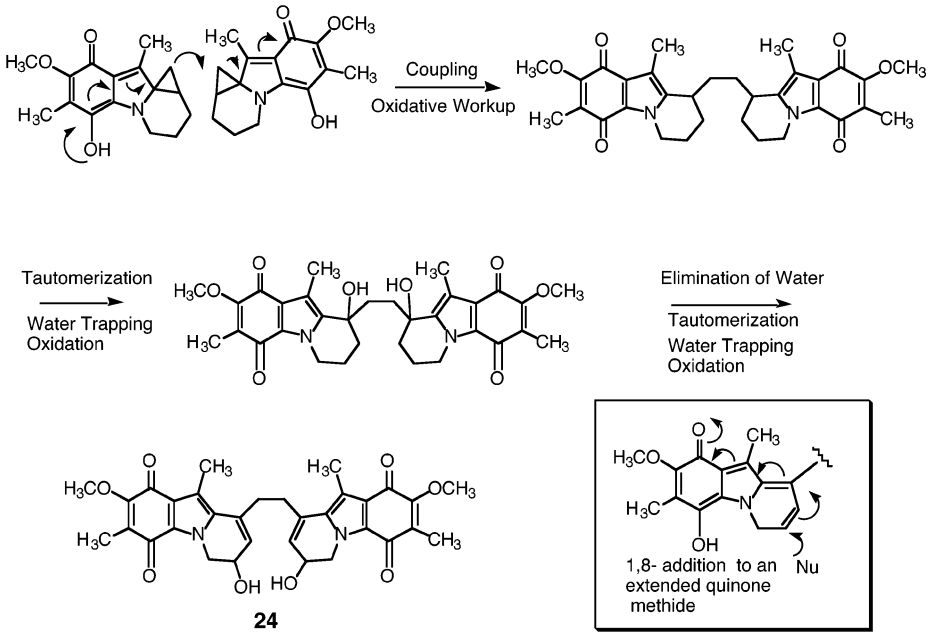
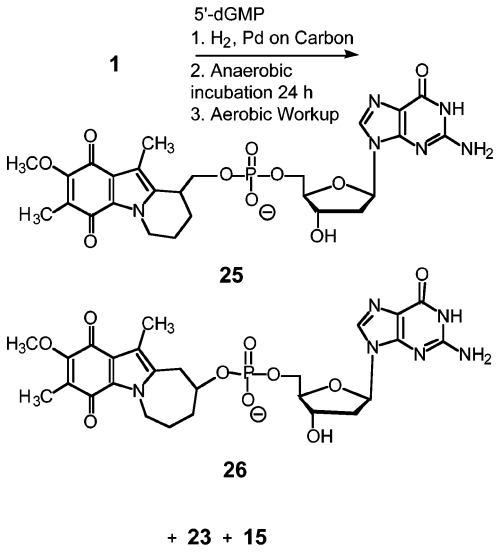
SCHEME 11



SCHEME 12



was evaporated in vacuo and the residue dissolved in 200 mL of ethyl acetate and extracted three times with 100 mL portions of saturated K_2CO_3. The organic layer was then washed with 100 mL of saturated NaCl solution, dried over Na_2SO_4, and finally filtered and evaporated to give a thick oily residue that solidified at room temperature. Recrystallization from ethanol gave light orange crystals: 4.9 g (92%) yield; mp 79−80 °C; TLC (chloroform) R_f = 0.47. IR (KBr pellet): 3086, 2966, 2849, 2243 (CN), 1574, 1500 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.97 (1H, s), 7.10 (1H, s), 4.95 (1H, q), 3.99 (3H, s), 2.26 (3H, s), 1.71 (3H, d). Anal. Calcd for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 60.06; H, 5.52; N, 12.80.

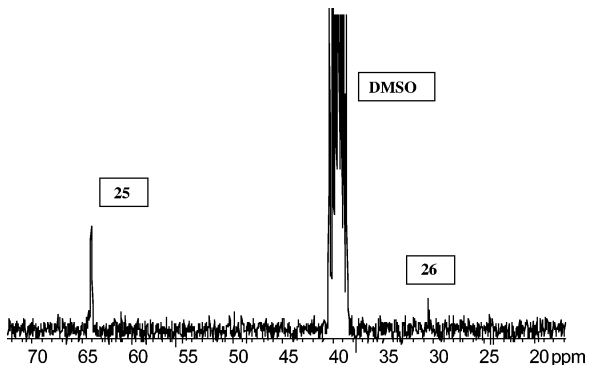


FIGURE 4. Enriched ^{13}C NMR of the GMP trapping products of reduced **1**.

A solution of 5.0 g (22.7 mmol) of methylated **8** in 200 mL of ethanol was degassed with nitrogen for 5 min. followed by the addition of 7.15 g of ammonium formate and 1 mL of acetic acid. To the resulting solution was added 1.5 g of 5% Pd on carbon, and the reaction was stirred for 45 min at 80 °C and then filtered through Celite. The solvent was evaporated in vacuo, and the solid residue was extracted with dichloromethane. The organic layer was washed with saturated NaCl solution, dried over Na_2SO_4, filtered, and the solvent was evaporated to afford the brown crystalline product: 3.1 g (78%) yield; mp 103−104 °C; TLC (chloroform) R_f = 0.49. IR (KBr pellet): 3383, 2927, 1472, 1207, 1062 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.64 (1H, s), 7.10 and 6.94 (2H, s), 6.87 (1H,d), 3.90 (3H,s), 2.33 (3H, s), 2.30 (3H, s). Anal. Calcd for $C_{11}H_{13}NO$: C, 75.40; H, 7.48; N, 7.99. Found: C, 75.17; H, 7.44; N, 7.99.

2-Methoxy-3,10-dimethyl-7,8,9a,10-tetrahydro-6*H*-pyrido[1,2-*a*]indol-9-one (10). Synthesis was carried out in two steps from compound **9** as follows: A stirred mixture of 2.0 g (11.41 mmol) of **9** in 30 mL of dimethylacetamide, 4.73 g of anhydrous K_2CO_3, and 2.0 mL of γ-butyrolactone was heated at reflux under N_2 for 48 h. The reaction mixture was diluted with H_2O, and the resulting solution was washed with toluene. The aqueous layer was acidified with concentrated HCl and then extracted with toluene. The extracts were evaporated, and the residue was purified by flash chromatography on silica gel using chloroform/ ethyl acetate (1:1) as the eluent. The isolated product was recrystallized from dichloromethane/hexane: 1.85 g (65%) yield; mp 117−118 °C; TLC (chloroform, 9:1) R_f = 0.62. IR (KBr pellet): 2931 (br), 1706, 1478, 1242 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.04 (1H, s), 6.91 (1H, s), 6.74 (1H, s), 4.08 (2H,t), 3.88 (3H,s), 2.34 (3H,s), 2.30 (2H,t), 2.27 (3H,s), 2.11 (2H,q).

A mixture of 1.5 g (6.06 mmol) of the above product and 6 g of polyphosphoric acid was heated at 90 °C for 1 h and then poured over ice. The resultant slurry was stirred for 4 h, diluted with H_2O, and extracted with Et_2O. The combined extracts were washed with

saturated aqueous $NaHCO_3$, dried with Na_2SO_4, filtered, and vacuum-dried. The residue was purified by flash chromatography on silica gel using dichloromethane as the eluent, and the isolated product recrystallized from dichloromethane/hexane: 1.35 g (91%) yield; mp 150−152 °C; TLC (chloroform/ethyl acetate, 90:10) R_f = 0.44. IR (KBr pellet): 2963, 2918, 1657, 1628, 1535, 1481, 1416, 1339, 1246, 1209 cm^{-1}. H NMR ($CDCl_3$): δ 7.07 and 6.94 (2H, 2s), 4.12 (2H, t, J = 6 Hz), 3.89 (3H, s), 2.68 (2H, t, J = 6 Hz), 2.65 (3H, s), 2.36 (3H, s), 2.32 (2H, p, J = 6 Hz). MS (EI mode) m/z: 243 (M^+) 228 ($M^+ - CH_3$), 214 ($M^+ - CH_2CH_3$), 200. Anal. Calcd for $C_{15}H_{17}NO_2$: C, 74.05; H, 7.04; N, 5.76. Found: C, 74.10; H, 7.04; N, 5.7.

6,7,8,9-Tetrahydro-3,10-dimethyl-2-methoxy-1-nitro-9-oxopyrido[1,2-*a*]indole (11). A mixture of 250 mg (1.03 mmol) of **10** and 25 mL of dichloromethane was cooled at 0 °C. To this mixture was added 0.20 mL of 69% nitric acid. After this mixture was stirred for 20 min, the reaction was diluted with 10 mL of saturated $NaHCO_3$, and the CH_2Cl_2 layer was separated and dried (Na_2SO_4). After removal of the solvent, the crude solid was purified by flash chromatography on silica gel using chloroform as the eluent. The isolated product was recrystallized from chloroform/hexane to afford the product as a yellow crystalline solid: 150 mg (51%) yield; mp 192−194 °C; TLC (chloroform/ethyl acetate, 90:10) R_f = 0.41. IR (KBr pellet): 2957, 1751, 1667, 1531, 1329, 1224 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.25 (1H, s), 4.18 (2H, t, J = 6 Hz), 3.87 (3H, s), 2.72 (2H, t, J = 6 Hz), 2.51 and 2.46 (6H, 2s), 2.35 (2H, d, J = 6 Hz). MS (EI mode) m/z: 288 (M^+), 271 ($M^+ - OH$), 255, 241, 227, 212. Anal. Calcd for $C_{15}H_{16}N_2O_4$: C, 62.49; H, 5.59; N, 9.72. Found: C, 62.55; H, 5.53; N, 9.73.

9-Cyano-6,7,8,9-tetrahydro-3,10-dimethyl-2-methoxy-1-nitropyrido[1,2-*a*]indole (12). A solution of 61 mg (0.21 mmol) of **11** and 124 mg (0.64 mmol) of tosylmethyl isocyanide in 2 mL of dry dimethoxyethane was cooled at 0 °C. To this solution was added a sodium ethoxide solution freshly prepared by adding 23 mg (1.04 mmol) of sodium to a mixture of 0.25 mL of dry ethanol and 0.35 mL of dry dimethoxyethane. The reaction mixture was stirred for 1 h at 0 °C and then for 1 h at room temperature. The reaction mixture was diluted with water and acidified with hydrochloric acid and then extracted three times with 25 mL portions of chloroform. The organic extracts were dried (Na_2SO_4) and concentrated to a residue, which was purified by flash chromatography on silica gel using chloroform as the eluent. The isolated product was recrystallized from chloroform/hexane: 30 mg (48%) yield; mp 154−156 °C; TLC (chloroform/ethyl acetate, 90:10) R_f = 0.49. IR (KBr pellet): 2953, 2924, 2236, 1526, 1462, 1041 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.19 (1H, s), 4.26 (1H, m), 4.22 and 3.81 (2H, 2m), 3.87 (3H, s), 2.45 (3H, s), 2.38 and 2.08 (2H, 2m), 2.31 and 2.22 (2H, 2m), 2.15 (3H, s). MS (EI mode) m/z: 299 (M^+), 282 ($M^+ - OH$), 266, 251, 237, 223. Anal. Calcd for $C_{16}H_{17}N_3O_3$: C, 64.20; H, 5.72; N, 14.04. Found: C, 64.13; H, 5.74; N, 13.97.

9-^{13}C-9-cyano-6,7,8,9-tetrahydro-3,10-dimethyl-2-methoxy-1-nitro-1*H*-pyrido[1,2-*a*]indole, 12*. A solution of 30 mg (0.1 mmol) of **12** and 20 mg (0.4 mmol) of $Na^{13}CN$ in 1 mL of dry DMSO was heated at 75 °C under N_2 for 3 h and then cooled to room temperature. To the reaction was added 10 mL of water, and the mixture was extracted three times with 25 mL portions of $CHCl_3$. The organic extracts were combined and dried with Na_2SO_4, concentrated to a residue, and then purified by flash chromatography on silica gel using chloroform as the eluant. The isolated **12*** was recrystallized from chloroform/hexance: 15 mg (50%) yield. Incorporation of ^{13}C in the cyanide group was 60% by mass spectrometry. ^{13}C NMR ($CDCl_3$): δ 118.5 ppm.

Methyl 6,7,8,9-Tetrahydro-3,10-dimethyl-2-methoxy-1-nitropyrido[1,2-*a*]indole-9-carboxylate (13). To a mixture of 151 mg (0.51 mmol) of **12** in 8 mL of methanol cooled to −20 to −40 °C was added hydrogen chloride gas until saturated. The resulting mixture was stoppered and stored in the freezer for 12 h and then in a refrigerator for 10 h. The reaction mixture was concentrated to a residue and combined with 10 mL of 3N HCl. This mixture was warmed to 70 °C for 10 min and then allowed to sit at room temperature for 24 h. The product was extracted three times with 25 mL portions of chloroform, and the extracts were dried (Na_2SO_4) and concentrated to a residue. The product was purified by flash chromatography on silica gel using chloroform as the eluant followed by recrystallization from chloroform/hexane: 120 mg (72%) yield; mp 85−87 °C; TLC (chloroform) R_f = 0.37. IR (KBr pellet): 2953, 2361, 1730, 1523, 1240, 1028 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.17 (1H, s), 4.20 and 3.78 (2H, 2m), 4.07 (1H, m), 3.86 (3H, s), 3.70 (3H, s), 2.43 and 2.03 (6H, 2s), 2.38 and 1.99 (2H, 2m), 2.25 and 2.04 (2H, 2m). MS (EI mode) m/z: 315 ($M^+ - 17$), 273, 226, 198, 182. Anal. Calcd for $C_{17}H_{20}N_2O_5$: C, 61.44; H, 6.07; N, 8.43. Found C, 61.53; H, 6.01; N, 8.42. ^{13}C NMR ($CDCl_3$): δ 172.8 ppm.

6,7,8,9-Tetrahydro-9-(hydroxymethyl)-3,10-dimethyl-2-methoxy-1-nitropyrido[1,2-*a*]indole(14). To 5 mL of dry THF, cooled to −20 °C, was added 120 mg (2 mmol) of LAH followed by addition of a solution of 150 mg of **13** in 5 mL of dry THF over a 5 min period. The reaction was then stirred for 15 min at −10 to −20 °C and then diluted with 5 mL of ethyl acetate. The mixture was filtered through Celite, concentrated to a residue, and purified by flash chromatography on silica gel using chloroform as the eluant. The isolated product was recrystallized from chloroform/hexane: 120 mg (91%) yield; mp 139−141 °C; TLC (chloroform/methanol, 90:10) R_f = 0.51. IR (KBr pellet): 3385, 2945, 2884, 2361, 1524, 1462, 1342, 1273, 1154, 1028 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.15 (1H, s, aromatic proton), 4.18 and 3.72 (2H, 2m), 3.86 (3H, s), 3.36 (1H, m), 2.43 (3H, s), 2.26 and 1.85 (2H, 2m), 2.11 and 2.02 (2H, 2m), 2.10 (3H, s). MS (EI mode) m/z: 304 (M^+), 287 ($M^+ - OH$), 273 ($M^+ - CH_2OH$), 256, 241, 226, 198. Anal. Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.20. Found: C, 62.90; H, 6.53; N, 9.14. ^{13}C NMR ($CDCl_3$): δ 64.0 ppm.

6,7,8,9-Tetrahydro-9-hydroxymethyl-3,10-dimethyl-2-methoxypyrido[1,2-*a*]indole-1,4-dione (15). This compound was prepared in two steps: A mixture of 100 mg (0.328 mmol) of **14**, 160 mg of 5% pd on carbon, 50 mL of methanol, and 5 drops of acetic acid was reduced for 2 h under 50 psi H_2. The catalyst was removed by filtration through Celite, and the mixture was concentrated to a residue. No attempt was made to purify the reactive amino alcohol product.

This product was dissolved in 10 mL of water containing 200 mg of monobasic potassium phosphate. To this solution was added a solution consisting of 500 mg of Fremy Salt in 30 mL of water containing 300 mg of monobasic potassium phosphate. The reaction mixture was stirred at room temperature for 18 h and then extracted four times with 50 mL portions of chloroform. The extracts were dried (Na_2SO_4), concentrated to a red solid, and subjected to silica gel thin-layer chromatographic separation using (1:1) chloroform/ethyl acetate as the eluent. The isolated product **15** was recrystallized from (chloroform/hexane): 10 mg (10%) yield; TLC (chloroform/methanol, 90:10) R_f = 0.40. IR (KBr pellet): 3451, 2922, 2853, 1657, 1632 cm^{-1}. ^{13}C NMR ($CDCl_3$) 179.95, 178.21, 156.16, 135.14, 129.37, 127.84, 122.24, 118.81, 64.01, 60.89, 45.74, 34.33, 21.72, 19.09, 10.10, 8.66. ^{1}HNMR ($CDCl_3$): δ 4.59 and 4.03 (2H, 2m), 3.96 (3H, s), 3.74 (2H, m), 3.18 (1H, m), 2.29 (3H, s), 2.18−1.82 (4H, m), 1.94 (3H, S). MS (EI mode) m/z: 289 (M^+), 258, 243, 215, 195. Anal. Calcd for $C_{16}H_{19}NO_4 \cdot 0.1CHCl_3$: C, 64.19; H, 6.39; N, 4.65. Found: C, 64.75; H, 6.58; N, 4.88. ^{13}C NMR ($CDCl_3$): δ 64.01 ppm.

6,7,8,9-Tetrahydro-9-hydroxymethyl-9-hydroxyl-3,10-dimethyl-2-methoxypyrido[1,2-*a*]indole-1,4-dione (16) and 6,7,-Dihydro-9-hydroxymethyl-3,10-dimethyl-2-methoxypyrido[1,2-*a*]-indole-1,4-dione (17). In addition to **15** the following compounds were isolated from the reaction by preparative TLC. 6,7,8,9-Tetrahydro-9-hydroxymethyl-9-hydroxyl-3,10-dimethyl-2-methoxypyrido[1,2-*a*]indole-1,4-dione (**16**): 4 mg (20% yield); TLC (chloroform/methanol, 90:10) R_f = 0.19. IR (KBr pellet): 3430, 2928, 1640 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 4.49 and 4.06 (2H, 2m, 6-diastereomeric methylene protons), 3.95 (3H, s, methoxy), 3.90

and 3.68 (2H, 2m, methylene protons), 2.46 and 1.92 (6H, 2s, 3, 10-dimethyl), 2.38 ~ 1.75 (4H, m, 7,8-diastereomeric methylene protons). Anal. Calcd for $C_{16}H_{19}NO_4 \cdot 0.1H_2O$: C, 62.57; H, 6.25; N, 4.55. Found C, 62.61; H, 6.26; N, 4.56. ^{13}C NMR ($CDCl_3$) δ 65.9 ppm.

6,7,-Dihydro-9-hydroxymethyl-3,10-dimethyl-2-methoxypyrido-[1,2-*a*]indole-1,4-dione (**17**): 1 mg (5%) yield; TLC (chloroform/ methanol, 90:10) $R_f = 0.38$. IR (KBr pellet): 3534, 2924, 1655, 1628 cm^{-1}; ^1H NMR ($CDCl_3$): δ 6.06 (1H, t, $J = 5.1$ Hz, 8-alkene proton), 4.53 (2H, s, methylene protons), 4.47 (2H, t, $J = 7.2$ Hz, 8-methylene protons), 3.99 (3H, s, methoxy), 2.53 and 1.95 (6H, 2s, 3, 10-dimethyl), 2.47 (2H, q, $J = 5.1$ Hz, 7-methylene protons). MS (EI mode) *m*/*z*: 287 (M^+), 272, 195, 153, 137, 116.

6,7,8,9-Tetrahydro-9-hydroxymethyl-9-hydroxyl-3,10-dimethyl-2-methoxypyrido[1,2-*a*]indol-1-imin,4-one(imino-16). This compound was prepared in two steps: A mixture of 100 mg (0.328 mmol) of **14**, 160 mg of 5% Pd on carbon, 50 mL of methanol was reduced for 2 h under 50 psi H_2.The catalyst was removed by filtration through Celite and concentrated to a residue. No attempt was made to isolate the reactive amino alcohol product. This product was dissolved in 3.0 mL acetone. To this a solution of phosphate buffer pH 7.2 with 700 mg of Fremy salt was added. The reaction mixture was stirred at room temperature for 15 min and then extracted four times with 50 mL of chloroform. The extracts were dried (Na_2SO_4), concentrated to a yellow solid, and subjected to silica gel thin-layer chromatographic separation using (1:1) chloroform/ ethyl acetate as the eluent. The isolated product imino-**16** was recrystallized from (chloroform/hexane): 52 (52%) yield; mp 179 > (dec); TLC (ethyl acetate) $R_f = 0.33$. IR (KBr pellet): 3414, 3242, 2936, 2860, 1639, 1616, 1587 cm^{-1}. ^{13}C NMR (DMSO): 177.13, 159.08, 153.49, 138.51, 124.88, 124.79, 122.92, 117.33, 70.65, 65.71, 61.31, 45.35, 31.64, 19.58, 11.76, 8.97. ^1H NMR (DMSO): δ 10.76 (1H, s), 5.16 (1H, s), 4.85 (1H, t), 4.29 and 4.14 (2H, 2m), 3.72 (3H, s), 3.62 and 3.53 (2H, 2m), 2.49 (3H, s), 2.21 (1H, m), 2.34 (2H, 2m), 1.88 (3H, s), 1.62 (1H, m). MS ($APCI^+$ mode) *m*/*z*: 305.1503. MALDI *m*/*z*: calculated, 305.150 (M + 1); found, 305.152 (M + 1). Anal. Calcd for $C_{16}H_{20}N_2O_4 \cdot 0.1CHCl_3$: C, 61.14; H, 6.41; N, 8.86. Found: C, 60.78; H, 6.34; N, 8.64.

6,7,8,9-Tetrahydro-3,10-dimethyl-9-[(methanesulfonoxy)-methyl]-2-methoxypyrido[1,2-*a*]indole-1,4-dione (1). To a stirred of solution of 15 mg of **15** in 0.3 mL of dry pyridine, cooled to 0 °C, was added 0.06 mL of methanesulfonyl chloride. After the reaction was stirred for 20 min at 0 °C and then 1.5 h at room temperature, the reaction mixture was diluted with a mixture consisting of 10 mL chloroform, and 10 mL of water was then added dropwise to decompose the excess methanesulfonyl chloride. The chloroform layer was washed consecutively with 2 N HCl, 5% sodium bicarbonate, and finally water. The chloroform layer was dried over Na_2SO_4 and then concentrated to an orange solid, which was recrystallized from chloroform/ hexane): 16 mg (84%) yield; mp 103−104 °C; TLC (chloroform/methanol, 90:10); $R_f = 0.69$. IR (KBr pellet): 3634, 2942, 1655, 1636, 1611, 1346, 1171 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.67 and 3.96 (2H, 2m), 4.31 and 4.18 (2H, 2m), 3.98 (3H, s), 3.46 (1H, m), 3.02 (3H, s), 2.30 (3H, s), 2.17 and 1.79 (2H, 2m), 2.00 (2H, m), 1.94 (3H, s). MS (EI mode) *m*/*z*: 367 (M^+), 271 ($M^+ - MsOH$), 258 ($M^+ - CH_2OMs$), 243, 228, 215. Anal. Calcd for $C_{17}H_{21}NO_6S$: C, 55.57; H, 5.76; N, 3.81. Found C, 55.55; H, 5.76; H, 3.81. ^{13}C NMR ($CDCl_3$): δ 68.4 ppm.

Hydrolysis of Reduced 1 in Anaerobic Methanol. To a mixture consisting of 15 mg (0.04 mmol) of **1** and 6 mg of 5% Pd on carbon was added 8 mL of methanol. The mixture was bubbled with argon for 15 min, with hydrogen gas for 10 min, and finally with argon for 15 min. The reaction mixture was incubated at 30 °C for 24 h and then combined with air and stirred for 1 h. The catalyst was filtered off, and the filtrate was concentrated to a red solid, which was subjected to preparative silica gel thin-layer chromatographic separation using chloroform as the eluant. The physical properties of products are provided below. **3,9,10-Trimethyl-2-methoxy-8,9-dihydropyrido[1,2-*a*]indole-1,4-dione (18).** A total of 0.3 mg (3%) yield; TLC (chloroform), $R_f = 0.42$. ^1H NMR ($CDCl_3$): δ 5.71 (1H, m, alkene proton), 4.43 (2H, t, $J = 7.2$ Hz, 6-diastereomeric methylene protons), 3.99 (3H, s, methoxy), 2.52, 2.16, 1.95 (9H, 3s, 3, 9, 10-trimethyl), 2.39 (2H, m, 7-diastereomeric methylene protons). MS (El mode) *m*/*z*: 271 (M^+), 256 ($M^+ - CH_3$), 241, 228. **3,9,10-Trimethyl-2-methoxy-6,7,8,9-tetrahydropyrido[1,2-*a*]indole-1,4-dione (19).** A total of 0.8 mg (7%) yield; TLC (chloroform), $R_f = 0.51$. ^1H NMR ($CDCl_3$): δ 4.57 (2H, m, 6-diastereomeric methylene protons), 3.96 (3H, s, methoxy), 3.13 (3H, m, methine proton), 2.27, 1.94 (6H, 2s, 3, 10-dimethyl), 2.04, 1.90, and 1.71 (4H, 3m, 7, 8-diastereomeric methylene protons), 1.25 (3H, t, $J = 7.2$ *Hz*). MS (El mode) *m*/*z*: 273 (M^+), 258 ($M^+ - CH_3$), 230, 215. **3,9,10-Dimethyl-2-methoxypyrido[1,2-*a*]indole-1,4-dione (20).** A total of 0.7 mg (6.5%) yield; TLC (chloroform), $R_f = 0.40$.^1H NMR ($CDCl_3$): δ 9.40 (1H, d, $J = 6$ Hz, aromatic proton from pyrido ring), 6.82 (1H, m, aromatic proton from pyrido ring), 3.98 (3H, s, methoxy), 2.85, 2.68 and 2.05 (9H, 3s, 3, 9, 10-trimethyl). MS (El mode) *m*/*z*: 269 (M^+), 254 ($M^+ - CH_3$), 226. **3,10-Dimethyl-2-methoxy-9-methoxymethyl-6,7,8,9-tetrahydropyrido[1,2-*a*]indole-1,4-dione (21).** A total of 2 mg (16%) yield; TLC (chloroform), $R_f = 0.29$. IR (KBr pellet): 2924, 1661, 1636, 1203, 1456 cm^{-1}. ^1H NMR ($CDCl_3$): δ 4.63 and 3.96 (2H, 2m, 6-diastereomeric methylene protons), 3.97 (3H, s, methoxy), 3.42 (2H, m, methylene protons), 3.37 (3H, s, methoxy), 3.26 (1H, s, methine proton), 2.29 and 1.94 (6H, 2s, 3, 10-dimethyl), 2.13, 2.02 and 1.76 (4H, 3m, 7, 8-diastereomeric methylene protons). MS (El mode) *m*/*z*: 303 (M^+), 258 ($M^+ - CH_2OCH_3$), 243, 226. **3,10-Dimethyl-2,9-dimethoxy-6,7,8,9-tetrahydroazepino[1,2-*a*]indole-1,4-dione (22).** A total of 2.5 mg (20%) yield; TLC (chloroform), $R_f = 0.28$. ^1H NMR ($CDCl_3$): δ 4.86 and 4.49 (2H, 2m, 6-diastereomeric methylene protons), 3.98 (3H, s, methoxy), 2.91 (2H, m, 10-diastereomeric methylene protons), 3.36 (3H, s, methoxy), 2.29 and 1.94 (6H, 2s, 3, 10-dimethyl), 2.05, 1,88 and 1.67 (4H, 3m, 7, 8-diastereomeric methylene protons). MS (El mode) *m*/*z*: 303 (M^+), 288 ($M^+ - CH_3$), 272, 258 ($M^+ - CH_2OCH_3$), 245.

Hydrolysis of $1H_2$ in Anaerobic Aqueous Buffer. A solution consisting of 0.5 mL DMSO and 5 mg (0.06 mmol) of **1** (^{13}C enriched) was added to 2 mL of 0.05 M pH 7.4 tris buffer containing 1 M KCl. To this solution was added 3 mg of 5% Pd on carbon, and the mixture was then degassed with argon for 30 min, followed by bubbling hydrogen gas for 15 min, and finally bubbling with argon for 30 min to remove the excess hydrogen. The reaction mixture was incubated at 30 °C for 24 h and then opened to the air. The catalyst was filtered off, and the filtrate extracted three times with 20 mL portions of chloroform. The extracts were dried (Na_2SO_4) and concentrated to a red solid, which was subjected to preparative silica gel thin-layer chromatographic separation using chloroform/methanol (95:5) as eluant. The physical properties of hydrolysis products are provided below.

6,7,8,9-Tetrahydro-3,10-dimethyl-9-[(methanesulfonoxy)-methyl]-2-methoxypyrido[1,2-a]indole-1,4-dione (1). A total of 0.1 mg (2%) yield. ^{13}C NMR ($CDCl_3$): δ 68.4 ppm.

6,7,8,9-Tetrahydro-9-hydroxymethyl-3,10-dimethyl-2-methoxypyrido[1,2-a]indole-1,4-dione (15). A total of 1.2 mg (30%) yield. ^{13}C NMR($CDCl_3$): δ 63.7 ppm.

3,10-Dimethyl-9-hydroxy-2-methoxy-6,7,8,9-tetrahydroazepino-[1,2-*a*]indole-1,4-dione (23). A total of 0.7 mg (18%) yield. ^{13}C NMR ($CDCl_3$): δ 32.4 ppm.

Dimer (24). A total of 0.4 mg (5%) yield. MS (El mode) *m*/*z*: 572, 556, 512, 496. ^{13}C NMR ($CDCl_3$): δ 44.8 ppm.

Preparation of the 5′-dGMP Adduct (25). To a solution of 35 mg (0.076 mmol) of the disodium salt of 5′-dGMP·$3H_2O$ in 5 mL of 0.05 M pH 7.4 tris·HCl, containing 5 mg of 5% Pd on carbon, was added a solution of 7 mg (0.14 mmol) of **1** in 0.5 mL of DMSO. The mixture was deaerated with argon for 30 min and then purged with H_2 gas until the reaction mixture became colorless, about 10

min. The excess H_2 was then removed by purging with argon for 10 min. The reduced reaction mixture was incubated at 30 °C for 24 h and then opened to the air. The reaction mixture was centrifuged at 12 000 g for 20 min, and the supernatant was extracted three times with chloroform to remove hydrolysis products. TLC analysis of the hydrolysis products revealed the presence of 6,7,8,9-tetrahydro-9-hydroxymethyl-3,10-dimethyl-2-methoxypyrido[1,2-a]indole-1,4-dione (**15**) (^{13}C NMR ($CDCl_3$): δ 63.7 ppm) and 3,10-dimethyl-9-hydroxy-2-methoxy-6,7,8,9-tetrahydroazepino[1,2-*a*]indole-1,4-dione (**23**) (^{13}C NMR ($CDCl_3$): δ 32.4 ppm).

The aqueous phase was orange in color and contained **25** and **26** along with unreacted 5′-dGMP. Concentration of the aqueous phase to complete dryness (no DMSO remaining) under high vacuum was followed by dissolution in ~1 mL of H_2O and then placement on a 100 g reverse phase column prepared with water. The 5′-dGMP moved somewhat faster than **25** and **26** while eluting with water. The orange band was collected and then concentrated to a dry residue and dissolved in 1 mL of water. The small amount of column residue was removed by centrifugation (12 000 g 10 min), and the supernant lyophilized to a red solid. ^{1}HMR (D_2O): δ 8.14 (1H, bs, amide proton from guanine), 7.90 (1H, s, C(8) proton from guanine), 6.56 (2H, bs, amine protons from guanine), 6.10 (1H, t, J = 7 Hz, C-1′ proton from deoxy-ribose), 4.43 (2H, m), 4.00 and 3.70 (2H, 2m), 3.83 (3H, s, methoxy), 3.80 (1H, s), 2.16 and 1.81 (6H, 2s). ^{13}C NMR (D_2O): δ 64.10 ppm

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