h. The catalyst was removed by filtration, the filtrate evaporated under reduced pressure, and the residual solid partitioned between 100 mL of chloroform and 50 mL of 2 N sodium carbonate solution. The organic phase was separated, dried over anhydrous magnesium sulfate, and evaporated to give a gum, which was chromatographed on silica gel. Compound **33** [0.25 g, 56%, mp 215–217 °C; NMR ($CDCl_3$) δ 1.25, 1.35 (2 t, 6 H, J = 6 Hz), 2.1–2.5 (m, 4 H), 2.55 (s, 3 H), 3.1 (s, 4 H), 4.15, 4.25 (2 q, 4 H, J = 6 Hz), 4.6–4.96 (m, 1 H), 7.05 (s, 1 H), 7.25, 7.75 (AB q, 4 H, J = 9 Hz), 8.35 (d, 1 H, J = 3 Hz), 8.77 (d, 1 H, J = 3 Hz); IR (Nujol) 3200, 3150, 1725, 1675, 1630, 1605 cm^{-1}. Anal. Calcd for $C_{27}H_{31}N_5O_7$: C, 60.32; H, 5.81; N, 13.03. Found: C, 59.98; H, 6.03; N, 12.92] was obtained upon elution with chloroform/methanol (97:3), while compound **34** [0.08 g, 18%, mp >200 °C; NMR ($CDCl_3$/Me_2SO-d_6) δ 1.24, 1.28 (2 t, 6 H, J = 6 Hz), 1.5–3.3 (m, 13 H), 2.18 (s, 3 H), 4.1, 4.18 (2 q, 4 H, J = 6 Hz), 4.4–4.7 (m, 1 H), 6.2 (s, 1 H), 7.28, 7.85 (AB q, 4 H, J = 9 Hz), 8.4 (d, 1 H, J = 8 Hz); IR (Nujol) 3320, 3250, 1730, 1630, 1575 cm^{-1}. Anal. Calcd for $C_{27}H_{35}N_5O_7$: C, 59.87; H, 6.51; N, 12.93. Found: C, 59.66; H, 6.71; N, 12.77] was obtained by elution with chloroform/methanol (95:5).

5,10-Dideazafolic Acid (3). A homogeneous solution of 0.175 g of **33** in 50 mL of methanol containing 3 mL of 1 N sodium hydroxide was stirred at room temperature for 72 h. Addition of 2 mL of acetic acid followed by centrifugation gave 0.125 g (86%) of **3** as a microcrystalline colorless solid: mp >200 °C; NMR (TFA-d_1) δ 2.3–2.7 (m, 2 H), 2.7–3.0 (m, 2 H), 3.25 (s, 5 H), 4.9–5.25 (m, 1 H), 7.35, 7.85 (AB q, 4 H, J = 9 Hz), 8.50 (s, 1 H), 8.90 (s, 1 H).

5,10-Dideaza-5,6,7,8-tetrahydrofolic acid (4) was obtained by alkaline hydrolysis of **34** as described above for the preparation of **3** from **33**: yielded 87%, mp >250 °C; NMR (TFA) δ 1.7–3.9 (m, 13 H), 5.0–5.25 (m, 1 H), 7.45, 7.85 (AB q, 4 H, J = 9 Hz).

5,10-Dideaza-9,10-didehydrofolic acid (35) was obtained in 29% yield by methanolic sodium hydroxide hydrolysis of **32** as described above for the preparation of **3** from **33**: mp >200 °C. The insolubility of **35** in all solvents, including TFA, precluded determination of its NMR spectrum.

Registry No. **1**, 95674-53-6; **2**, 95674-54-7; **3**, 85597-18-8; **4**, 95693-76-8; **7**, 7377-04-0; **8**, 95674-55-8; **9**, 95674-56-9; **10**, 72313-37-2; **11**, 95674-57-0; **12**, 95674-58-1; **13**, 95674-59-2; **14a**, 95674-60-5; **14b**, 95674-61-6; **15**, 87373-60-2; **16**, 88553-19-9; **17**, 95693-77-9; **18a**, 95674-62-7; **18b**, 95693-78-0; **19a**, 95674-63-8; **19b**, 95674-64-9; **20**, 95674-65-0; **21**, 95674-66-1; **22**, 95674-67-2; **23**, 95674-68-3; **24**, 80360-04-9; **25**·Br^-, 70583-34-5; **26**, 95674-69-4; **27**, 95693-79-1; **28**, 95674-70-7; **29**, 95674-71-8; **30**, 95674-72-9; **31**, 95674-73-0; **32**, 95674-74-1; **33**, 95674-75-2; **34**, 95674-76-3; **35**, 95693-80-4; malononitrile, 4341-85-9; 4-(ethoxycarbonyl)benzaldehyde, 6287-86-1; 4-(*tert*-butoxycarbonyl)benzaldehyde, 65874-27-3; guanidine, 113-00-8; diethyl L-glutamate hydrochloride, 1118-89-4; diethyl *N*-[4-(bromomethyl)benzoyl]glutamate, 70583-33-4; di-*tert*-butyl L-glutamate hydrochloride, 32677-01-3; dihydrofolate reductase, 9002-03-3; thymidylate synthetase, 9031-61-2; folate polyglutamate synthetase, 63363-84-8.

Synthesis and Biological Activity of 6-Substituted Mitosene Analogues of the Mitomycins[1]

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A series of 1-acetoxymitosene analogues, in which the substituent at C-6 was varied, was prepared by total synthesis and screened for activity against P388 leukemia in mice and induction of λ phage in *Escherichia coli*. Among the 6-substituents prepared, none was as effective as the methyl group in conferring biological activity. However, certain *N*-methylcarbamates were more active than the unsubstituted carbamates.

Previous studies on the relationship between structural modifications in mitomycin analogues and their antitumor activity have included variations in the 7-substituent (quinone ring), carbamate, and the aziridine ring.[2-8] One structural feature not investigated thus far is the 6-substituent in the quinone ring, which always has been a methyl group in analogues with the complexity of pyrrolo[1,2-*a*]indoles. This feature had been explored in simpler indole analogues to see its influence on antibacterial activity.[9,10] However, it has been shown that antibacterial activity does not correlate with antitumor activity in mitomycins.[11] Furthermore, none of the indole analogues showed antitumor activity,[12] although some relatively simple pyrrolo[1,2-*a*]indole analogues (e.g., **15**) are active.[8] For these reasons, it seemed desirable to conduct a study on the synthesis and antitumor activity of pyrrolo[1,2-*a*]indole analogues (mitosenes), based on the structure of **15**, but with different substituents in place of the 6-methyl group.

Chemistry. The synthesis of analogues of **15** presents a problem in that modification of the 6-methyl group has not been accomplished. Therefore, we decided to prepare first the 6-unsubstituted analogue **11** and then introduce novel substituents at C-6. Two routes for the synthesis of **11** were explored. One was based on well-established chemistry,[8,13,14] starting from ethyl 5-methoxyindole-2-

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(1) Taken, in part, from the Ph.D. Dissertation submitted to the Graduate College of the University of Arizona by Michael L. Casner, 1984.
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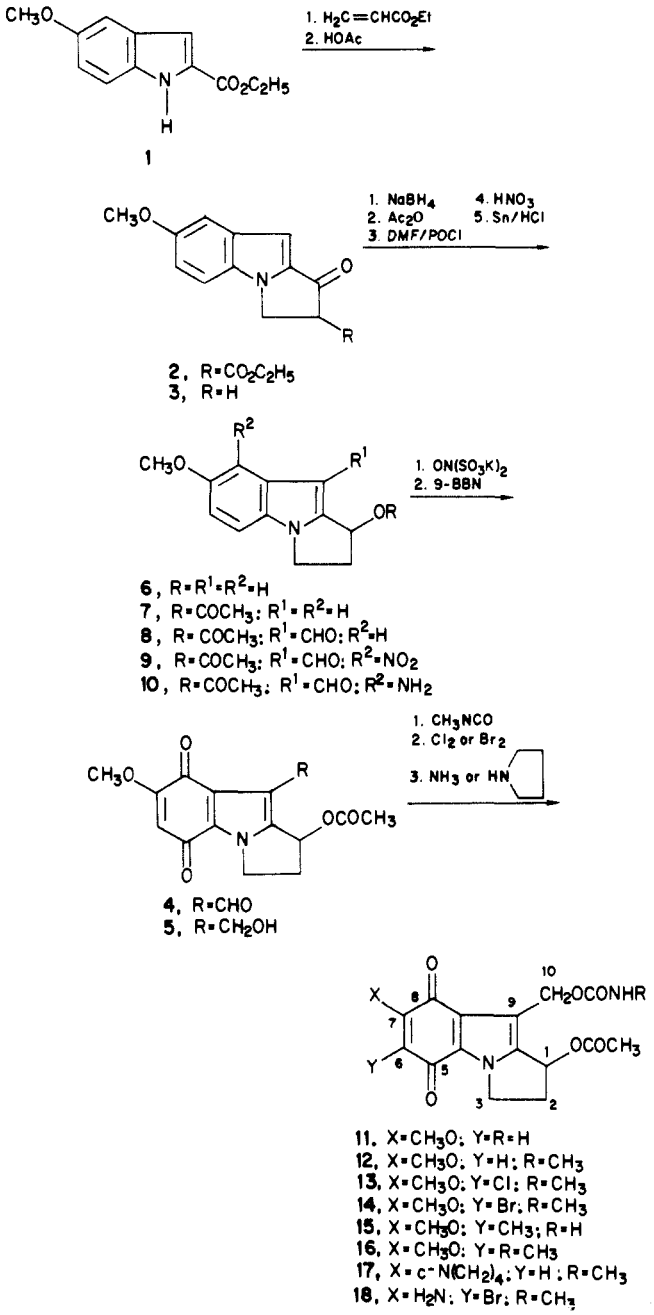
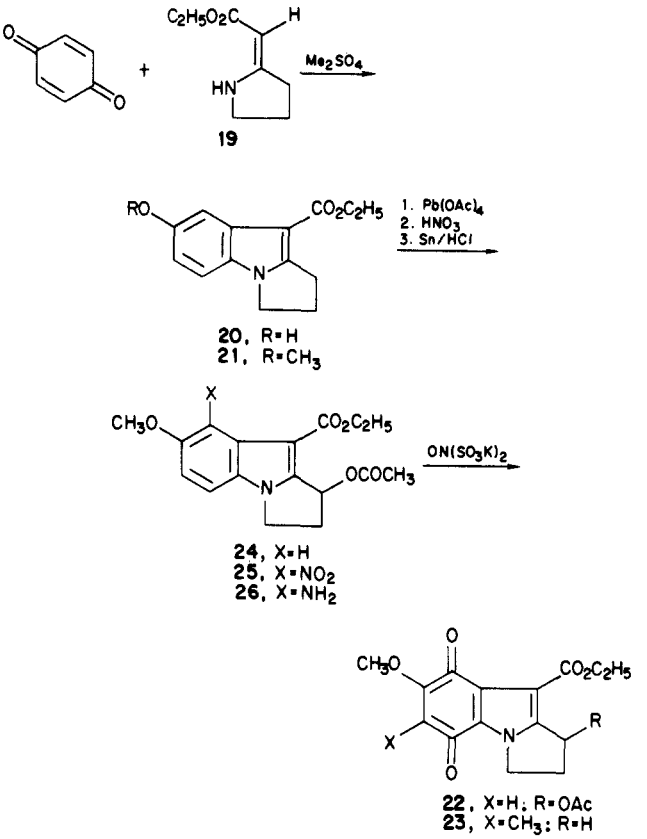
Scheme I



Scheme II



carboxylate (Scheme I), and it was successful. The other was based on a Nenitzescu synthesis[15] and Kametani's acetoxylation[16] (Scheme II). This route could not be carried beyond compound 22; however, a report of the conversion of a closely related compound (23) to a mitosene[17] (see below) suggests that the route might have succeeded if it had been studied exhaustively.

The pyrrolo[1,2-*a*]indole system was constructed from 1 by standard procedures involving condensation with ethyl acrylate and decarbethoxylation in refluxing acetic acid.[13,14] Reduction of 3 with sodium borohydride gave an alcohol 6, which was acetylated and treated with the Vilsmeier reagent to give aldehyde 8. This aldehyde was converted into the corresponding quinone 4 by the usual sequence of nitration, reduction, and Fremy's salt oxidation. Reduction of 4 to the corresponding 9-hydroxymethyl compound 5 provided the first problem in the synthesis. The original method for effecting this kind of transformation involved sodium borohydride reduction followed by reoxidation of the resulting hydroquinone with ferric chloride.[13,14] However, in the synthesis of 15 we found that this method gave partial replacement of the 1-acetoxy group by hydride. This reaction did not occur when we used 9-borabicyclononane followed by hydrogen peroxide.[8] Unfortunately, application of this procedure to 4 caused partial destruction of the molecule (probably oxidation initiated at the 6-position) and the yield of 5 was low. Returning to the borohydride reduction method improved the yield of 5, but it remained low. Introduction of a carbamate group by the usual method of phenyl chloroformate followed by ammonia also presented a problem. It was useful for the conversion of 5 to 11 (64% yield), despite the formation of some amino quinone byproduct. Concern for side reactions in treating anticipated halo quinones with ammonia led us to investigate the *N*-methylcarbamate analogue 12 as an alternative reference compound to 11. Analogue 13 was made in 80% yield by treating 5 with methyl isocyanate, and when it proved biologically active (see below), we decided to base the series of 6-substituted compounds on it.

A variety of reactions were investigated for the introduction of substituents at C-6. Some of them were successful and some were disappointing. The 6-chloro analogue 13 was obtained in low yield by treating 12 with chlorine in acetic acid, followed by reduction with sodium bisulfite. This reduction was necessary because an unstable intermediate that appeared to be an *N*-chlorocarbamate was isolated initially. Bromination of 12 in acetic acid was more successful and the product 14 was obtained in 60% yield. Compound 12 was treated with ammonia in an attempt to prepare a 6-amino-7-methoxy

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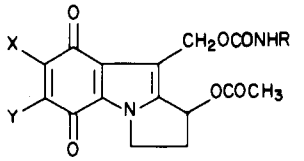
Table I. Biological Activity and Partition Coefficients of 6-Substituted Mitosenes



compd	X	Y	R	log P obsd[b]	log P calcd[c]	ILB:[a] ratio of plaques at 3.1 μg/mL	ILB:[a] ratio of plaques at 0.8 μg/mL	P388 mouse leukemia: % T/C at OD[d]
11	CH_3O	H	H	0.72	0.98	18.6	11.9	111
12	CH_3O	H	CH_3	1.19	1.46	10.1	6.0	106
13	CH_3O	Cl	CH_3	2.08	2.17	27.0	12.1	
14	CH_3O	Br	CH_3		2.32	12.4	7.1	100
15	CH_3O	CH_3	H	1.54[e]	1.54	24.5	13.2	165
16	CH_3O	CH_3	CH_3	2.39	2.02	34.1	16.6	180
17	c-$N(CH_2)_4$	H	CH_3	1.40	2.66	1.0	1.0	
18	H_2N	Br	CH_3	1.15	1.11	1.1	1.2	

[a] Determined at Bristol-Myers Co., Syracuse, NY. The ratio represents plaques induced in the treated culture to those appearing in the control. For a detailed description of this assay, see Price, K. E.; Buck, R. E.; Lein, J. *Appl. Microbiol.* **1964**, *12*, 428. [b] Distribution between 1-octanol and pH 7.4 7.4 phosphate buffer, according to the method of Hansch et al.: Hansch, C.; Muir, R. M.; Fujita, T.; Malongy, P. P.; Geiger, F.; Struch, M. J. *J. Am. Chem. Soc.* **1963**, *85*, 2817. [c] Estimated from the value measured for **15** and substituent constants taken from Hansch, C.; Leo, A. "Substituent Constants for Correlation Analysis in Chemistry and Biology", Wiley-Interscience: New York, 1979. [d] See Table II for a description of this assay. OD = optimal dose (maximum % T/C). [e] Value taken from Remers, W. A.; Schepman, C. S. *J. Med. Chem.* **1974**, *17*, 729.

quinone. However, the methoxy group was replaced and the product was 7-amino-6-bromo quinone **18**. An analogous transformation had been observed in the earlier indoloquinone analogues.[9] Alternative approaches to the preparation of 6-amino-7-methoxy quinones also failed. Treatment of 6-unsubstituted analogue **12** with amines appeared to give displacement of the methoxy group rather than addition at C-6. A characterized example of this process is the 7-pyrrolidino analogue **17**. Compound **12** did not give useful reactions with sodium azide, hydrazoic acid, or iodine azide, reagents that proved useful for the introduction of amino or azido groups in other systems, such as streptonigrin.[18] The 6-methyl analogue bearing an *N*-methylcarbamate **16** was prepared by treating the known 9-hydroxymethyl compound[8] with methyl isocyanate.

Synthesis of **11** or **12** by the route outlined in Scheme II appeared to offer a shortened sequence. The known *Z* isomer of ethyl (2-pyrrolidinylidene)acetate (**19**)[19] was condensed with 1,4-benzoquinone in a procedure based on one used by Yamada for the corresponding reaction with toluquinone.[15] Product **20** was converted to its methyl ether **21** and then treated with lead tetraacetate to give the 1-acetoxy derivative **24**.[16] Nitration, followed by reduction and Fremy's salt oxidation, converted **24** into the quinone **22**. At this point, conversion of **22** into **11** or **12** required only reduction of the carboxylate group to an hydroxymethyl group, followed by carbamate formation. It was hoped that conditions could be found in which the 1-acetoxy group was not cleaved; however, this cleavage would not be fatal, because it had been shown previously that selective reacetylation could be accomplished. The direct reduction of nitro derivative **25**, followed by Fremy's salt oxidation and carbamate formation, offered an even more direct potential route to **11** or **12**. In the outcome, it was not possible to find a suitable method for reducing either **22** or **25**. A variety of metal hydrides, diborane, and alane were tried. Although none of the product mixtures from these reductions was characterized fully, it appeared that cleavage of the 1-acetoxy group and saturation of the 9,9a-double bond had occurred in some of them. For example, an unstable product isolated from the treatment of **25** with $LiAlH_4$ (10% yield) showed the presence of an *unconjugated* ethyl ester (1725-cm^{-1} IR band) and a nitro group (1580 and 1360 cm^{-1}) but no acetoxy group (NMR evidence). A molecular ion at m/e 322 supported a structure based on these transformations. There is literature precedent for conjugate addition of hydride to α,β-unsaturated esters.

Although our attempts at reduction of **22** were unsuccessful, in an elegant mitosene synthesis, Coates and MacManus achieved the reduction of a closely related compound (**23**) by the two-step process of zinc and HCl followed by $LiAlH_4$.[17] It is possible that this process would work with **22**, but we had not material left to try it after their communication appeared.

Biological Activity. Although the *N*-methyl derivative **16** of our lead compound **15** showed comparable activity against P388 leukemia (Table II), the first three new 6-substituted mitosenes prepared (**11**, **12**, and **14**) were inactive in this assay. Consequently, we decided to substitute a more sensitive assay as the primary screen for biological activity. Induction of phage in *Escherichia coli* (ILB) was chosen for this purpose. In this assay, potential antitumor agents are found to activate latent phage so that it is expressed, resulting in the death of the host bacterium. The ratio of plaques (clear areas) in treated bacterial cultures to those in controls at specific drug concentrations has been found to correlate with the antitumor activity of proven clinical agents.

Table I shows the ratio of plaques formed at two different concentrations of the 6-substituted mitosenes. As a comparison, mitomycin C is active in the ILB assay at concentrations as low as 0.006 μg/mL. On the basis of the known lead compound **15**, it appears that improvement in ILB activity was obtained only in new analogue **16** and possibly **13**. These analogues have *N*-methylcarbamates and methyl and chloro substituents at C-6. All other analogues, except **11**, showed significant decreases in activity. Thus, it appears that the naturally occurring methyl group is the best substituent of any tried thus far at C-6, with the chloro substituent, which is similar in size and lipophilicity, as a close second. Complete inactivity was shown by analogues with amine substituents in the quinone ring. This kind of behavior was noted previously for indoloquinones[9] and aziridinomitosenes.[2] It probably re-

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Table II. Activity of Compounds against P388 Leukemia in Mice[a]

compd	dose, mg/kg per inj	effect MST (% T/C)	av wt change, g
16	25.6	180	−0.2
	12.8	145	0.0
	6.4	135	1.2
	3.2	120	1.0
15	6.4	165	0.1
	3.2	133	0.3
	1.6	124	0.6
	0.8	114	1.0
Mit C[b]	4.8	205	−2.2
	3.2	195, 229	−0.7, 0.5
	1.6	150, 186	−0.2, −0.4
	0.8	135, 176	0.5, −0.4
	0.4	130, 138	0.3, 0.3
	0.2	120, 133	0.8, 0.8

[a] Determined at Bristol-Myers Co., Syracure, NY. A tumor inoculum of 10^6 ascites cells was implanted in CDF_1 female mice. Six mice were used at each dose of the compound, given on day 1 only, and 10 control mice were injected with saline. MST = median survival time. Compounds considered active have % T/C > 125. There were no 30-day survivors with 16 or 15; however, mitomycin C gave two tumor-free survivors at the highest dose in each experiment. [b] For each compound, 16 and 15, a mitomycin C standard was run at the same time. The first column gives the results of the standard with 16 and second column gives the results with 15. Controls lived 10.5 and 10.0 days, respectively.

flects the difficulty for biological systems to reduce such compounds to their activated hydroquinone forms.

No statistically significant correlation could be made between log *P* and ILB activity for the family of compounds in Table I.

The activity of compound **16** against P388 leukemia in mice is given in greater detail and compared with that of **15** and mitomycin C in Table II. This table shows that **16** is less potent (minimum effective dose) than **15**, but it gives greater prolongation of life. Both mitosenes are less potent and efficaceous than mitomycin C.

Experimental Section

Melting points were determined on a Laboratory Instruments Mel-Temp and are corrected. IR spectra were taken on a Beckman IR-33 spectrometer with samples prepared as KBr pellets or as a film on NaCl plates. NMR spectra were taken routinely on a Varian EM-360L 60-MHz spectrometer or for higher resolution on a JEOL FX 90Q 90-MHz spectrometer. Absorptions are reported as ppm downfield from tetramethylsilane. Mass spectral data were obtained by direct probe with electron-impact ionization on a Varian 311A instrument. Thin-layer chromatography (TLC) was carried out with commercial 250-μm silica gel GF plates from Analtech. The phase "column chromatography on silica gel" denotes the use of MN silica gel 60 (0.05–0.2 mm) with gravity elution. The phrase "preparatory silica gel plate" indicates the use of silica gel 60 254 PF (from E. Merck) in approximately 1.5-mm thickness on a 20 × 20 cm glass plate. Elemental analyses were performed by the University of Arizona Analytical Center.

2,3-Dihydro-7-methoxy-1-oxo-1*H*-pyrrolo[1,2-*a*]indole (3). A stirred solution of 1 (30 g, 137 mmol) in 1.5 L of toluene under N_2 was treated with sodium hydride (8.0 g of 50% emulsion in mineral oil, 167 mmol). Ethyl acrylate (16.3 mL, 150 mmol) was added and the mixture was heated at reflux. Additional portions of ethyl acrylate were added after 1 h (1 mL) and 2 h (2 mL) and more sodium hydride suspension (3.65 g) was added after 2 h. After a total time of 6 h, the mixture was quenched with ethanol and treated with water, dilute HCl, and methylene chloride. The organic phase was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give crude **2** (mp 180–190 °C dec), which was used directly in the next step.

A solution of crude **2** in 2.0 L of acetic acid was treated with 110 mL of water and heated at reflux under N_2 for 16 h. The resulting dark solution was cooled and concentrated under reduced pressure, and the residue was treated with water and methylene chloride. The organic layer was washed with $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated. Purification of the residue by column chromatography on silica gel with a mobile phase consisting of methylene chloride to which an increasing porportion of ethyl acetate was added yielded 18.5 g (67.4%) of **3** as a pale yellow solid: mp 170 °C; NMR ($CDCl_3$) δ 7.1 (m, 4 H), 4.4 (t, 2 H), 3.85 (s, 3 H), 3.2 (t, 2 H); IR 1690, 1620 cm^{-1}. Anal. ($C_{12}H_{11}NO_2 \cdot 0.15H_2O$) C, H; N: calcd, 6.86; found, 6.37.

1-Acetoxy-2,3-dihydro-7-methoxy-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (8). A solution of **3** (15.8 g, 78.6 mmol) in 1 L of methanol was stirred with sodium borohydride (2.9 g, 78.6 mmol). After 1 h, dilute H_2SO_4 and methylene chloride were added. The organic layer was washed with $NaHCO_3$ solution and brine, dried, and concentrated to give 15.3 g of alcohol **6** (mp 105 °C), which was used directly in the next step.

A solution of crude **6** (15.2 g) in 75 mL of pyridine, under N_2, was cooled in an ice bath and treated with 50 mL of acetic anhydride. After 15 h, ice and methylene chloride were added. The organic layer was dried over Na_2SO_4, filtered, and concentrated. Because the crude product contained pyridine it was dissolved in methylene chloride, washed with dilute HCl, $NaHCO_3$, and brine, dried, and reconcentrated. The crude acetate **7** (17.2 g) [mp 110 °C; NMR ($CDCl_3$) δ 7.1 (m, 3 H), 6.3 (s, 1 H), 6.1 (dd, 1 H), 4.0 (m, 2 H), 3.85 (s, 3 H), 2.8 (m, 2 H), 2.1 (s, 3 H); IR 1720 cm^{-1}] was too unstable for purification by chromatography. It was used directly in the next step.

The Vilsmeier reagent was prepared by stirring DMF (10 mL, 130 mmol) and freshly distilled $POCl_3$ (7 mL, 75 mmol) under N_2 at ice-bath temperature for 33 min. A solution of **7** (16 g) in 25 mL of pyridine and 100 mL of methylene chloride was added and the mixture was stirred for 3 h. Cold, saturated sodium acetate solution was added and the mixture was stirred 12 h and then extracted with methylene chloride. The organic layer was dried over Na_2SO_4 and concentrated to an oil (20 g), which was purified by column chromatography on silica gel with use of a mobile phase consisting of methylene chloride to which an increasing proportion of ethyl acetate was added. This procedure gave 11.8 g (66.4%) of **8** as a pale yellow solid: mp 112 °C; NMR ($CDCl_3$) δ 10.5 (s, 1 H), 7.8 (d, 1 H), 7.25 (m, 1 H), 6.95 (m, 1 H), 7.0 (dd, 1 H), 6.45 (m, 2 H), 3.9 (s, 3 H), 3.1 (m, 2 H), 2.1 (s, 3 H); IR 1735, 1645 cm^{-1}. Anal. ($C_{15}H_{15}NO_4$) C, H, N: calcd, 5.12; found, 4.62.

1-Acetoxy-2,3-dihydro-7-methoxy-8-nitro-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (9). A solution of **8** (5.95 g, 21.8 mmol) in 125 mL of acetic acid was treated with 5 mL of concentrated nitric acid. After 1 h the mixture was cooled in an ice bath and treated with ice water. The resulting precipitate was washed with cold water and dried in the air. This procedure gave 5.95 g (86%) of **9** as a yellow solid: mp 169 °C; NMR ($CDCl_3$) δ 9.8 (s, 1 H), 7.1 (m, 2 H), 6.45 (dd, 1 H), 4.25 (m, 2 H), 3.9 (s, 3 H), 3.0 (m, 2 H), 2.1 (s, 3 H); IR 1750, 1670, 1535 cm^{-1}. Anal. ($C_{15}H_{14}N_2O_6$) C, H, N.

1-Acetoxy-2,3-dihydro-5,8-dioxo-7-methoxy-1*H*-pyrrolo-[1,2-*a*]indole-9-carboxaldehyde (4). A stirred suspension of **9** (5.95 g, 18.7 mmol) in 500 mL of 95% ethanol was treated with 100 mL of 1 N HCl and 9 g of tin metal (100 mesh). After 2 h, $NaHCO_3$ solution was added and the mixture was extracted with methylene chloride. This extract was washed with brine, dried over Na_2SO_4, and concentrated to give 5.95 g of crude amine **10** (mp 132–134 °C), which was used directly in the next step.

A solution of 5.95 g of crude amine **10** in 200 mL of acetone was treated with a suspension of potassium nitrosodisulfonate (15 g) in 225 mL of 0.3 M KH_2PO_4 (titrated to pH 6.1 with KOH solution). A second 5.2-g portion of potassium nitrosodisulfonate was added after 3 h. The mixture was stirred 2 more h and then extracted with methylene chloride. This extract was washed with $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography on silica gel with a mobile phase consisting of methylene chloride to which an increasing proportion of ethyl acetate was added. From the main yellow band was obtained 2.5 g (44%) of **4** as yellow solid: mp 189 °C after recrystallization from chloroform; NMR ($CDCl_3$) δ 10.4 (s, 1 H), 6.25 (dd, 1 H), 5.65 (s, 1 H), 4.5 (m, 2 H), 3.8 (s, 3 H), 2.9 (m, 2 H), 2.25 (s, 3 H); IR 1745, 1690, 1640 cm^{-1}. Anal. ($C_{15}H_{13}NO_6 \cdot 0.7CHCl_3$) C, H, N.

1-Acetoxy-2,3-dihydro-5,8-dioxo-9-(hydroxymethyl)-7-methoxy-1*H*-pyrrolo[1,2-*a*]indole (5). Method A. A solution of **4** (2.2 g, 7.2 mmol) in 500 mL of tetrahydrofuran, under N_2, was treated with 9-borabicyclo[3.3.1]nonane (40 mL of a 0.5 M solution in tetrahydrofuran). After 2 h the solution was cooled in an ice bath and treated with 3% hydrogen peroxide solution (containing 2.9 mmol of H_2O_2). Water and ether were added and the layers were separated. The water layer was extracted with methylene chloride and this extract was combined with the organic layer. The combined organic solution was dried over Na_2SO_4 and concentrated. Purification of the residue by chromatography on silica gel using methylene chloride containing 5% acetone as the solvent gave 2.46 g (34%) of **5** as a yellow solid: mp 174 °C; NMR ($CDCl_3$) δ 6.0 (dd, 1 H), 5.6 (s, 1 H), 4.65 (d, 2 H), 4.3 (m, 3 H), 3.9 (s, 3 H), 2.7 (m, 2 H), 2.1 (s, 3 H); IR 3550–3360, 1740, 1710, 1680 cm^{-1}. Anal. ($C_{15}H_{15}NO_6$) C, H, N.

Method B. A solution of **4** (1.2 g, 3.9 mmol) in 125 mL of absolute ethanol and 400 mL of tetrahydrofuran was treated with 272 mg (7.2 mmol) of sodium borohydride under N_2. After 2.5 h, another 272 mg of sodium borohydride was added. The mixture was stirred for 4 h and then treated with 20 mL of acetone. After 15 min, a solution of 3.65 g of potassium nitrosodisulfonate in pH 6.1 phosphate buffer was added and the mixture was extracted with methylene chloride. This extract was washed with $NaHCO_3$ solution, dried over Na_2SO_4, and concentrated. Purification of the residue by chromatography as described in method A gave 0.5 g (41%) of **5** as a yellow solid, which was identical in NMR and IR spectra with the sample prepared by method A.

1-Acetoxy-6-desmethyl-7-methoxymitosene (11). A solution of **5** (368 mg, 1.2 mmol) in 50 mL of methylene chloride and 25 mL of pyridine was stirred under argon at 0 °C and treated with phenyl chloroformate (2.5 mL, excess). The mixture was stirred 12 h and then treated with ice water and methylene chloride. The organic layer was washed with dilute HCl and brine, dried over Na_2SO_4, and concentrated to give the oily phenyl carbonate ester, which was used directly in the next step.

A solution of the phenyl carbonate in 50 mL of methylene chloride was placed in a flask fitted with a dry ice–acetone filled condenser. The flask was cooled in a dry ice–acetone bath and 50 mL of liquid ammonia was added. After 3 h, the ammonia and solvent were evaporated, and the oily residue was chromatographed on a column of silica gel with methylene chloride containing 45% ethyl acetate as solvent. This procedure gave 270 mg (64%) of **11** as an orange solid which was homogeneous on TLC in methylene chloride–acetone and in methylene chloride–ethyl acetate: mp 210 °C; NMR ($CDCl_3$) δ 6.3 (dd, 1 H), 5.7 (s, 1 H), 5.3 (s, 2 H), 4.9 (m, 2 H), 4.3 (m, 2 H), 3.8 (s, 3 H), 2.9 (m, 2 H), 2.8 (m, 2 H), 2.1 (s, 3 H); IR 3420, 3310, 1760, 1715, 1625 cm^{-1}; HRMS, m/z (M, $C_{16}H_{16}N_2O_7$) calcd 348.0957, found 348.0939.

1-Acetoxy-6-desmethyl-7-methoxy-*N*-methylmitosene (12). A solution of **5** (220 mg) in 25 mL of methylene chloride was treated with triethylamine (1 mL) and methyl isocyanate (1 mL, excess). After 16 h, the mixture was treated with water and the organic layer was washed with brine, dried over Na_2SO_4, and concentrated. Purification of the residue on a column of silica gel with a mobile phase consisting of methylene chloride to which an increasing proportion of ethyl acetate was added gave 210 mg (80%) of **12** as an orange solid, which had mp 210 °C after recrystallization from acetone: NMR ($CDCl_3$) δ 6.3 (dd, 1 H), 5.7 (s, 1 H), 5.3 (s, 2 H), 4.9 (m, 1 H), 4.3 (m, 2 H), 3.8 (s, 3 H), 2.9 (m, 2 H), 2.8 (d, 3 H), 2.1 (s, 3 H, 6 H); IR 3400, 1730, 1670, 1640 cm^{-1}. Anal. ($C_{17}H_{18}N_2O_7 \cdot C_3H_6O$) C, H, N; calcd, 5.75; found, 5.30.

1-Acetoxy-6-chloro-6-desmethyl-7-methoxy-*N*-methylmitosene (13). A stirred solution of **12** (191 mg) in acetic acid (100 mL) was treated with Cl_2 gas until the color changed from orange to pale yellow-green (15 s). The solution was flushed with argon and treated with sodium acetate. After 3 h, water and methylene chloride were added. The organic layer was washed with $NaHCO_3$ solution, dried over Na_2SO_4 and Na_2CO_3, and concentrated to give 72 mg of solid. This solid appeared to be the *N*-chlorocarbamate, because it showed no NH proton in its NMR or IR spectra. It was dissolved in methylene chloride and shaken with 5% sodium bisulfite solution. The organic layer was dried over Na_2SO_4 and concentrated. Purification of the residue by chromatography on a preparative-layer plate with methylene chloride containing 5% acetone gave 18 mg (8.5%) of **13** as an orange solid, which also was homogeneous on chromatography with methylene chloride–ethyl acetate as solvent: mp 160 °C dec; NMR ($CDCl_3$) δ 6.2 (dd, 1 H), 5.2 (s, 2 H), 4.8 (s, 1 H), 4.3 (m, 1 H), 4.2 (s, 3 H), 3.7 (br s, 2 H), 2.75 (d, 3 H), 2.6 (m, 2 H), 2.1 (s, 3 H); IR 3400, 1740–1700, 1660 cm^{-1}; HRMS, m/z $C_{15}H_{10}NO_6Cl$ (M – CH_3NCO) calcd 339.0494, found 339.0510, and $C_{13}H_{13}NO_4Cl$ (M – CH_3NCO – HOAc) 279.0299, found 279.0242. The molecular ion was not detectible. Anal. ($C_{17}H_{17}N_2O_7Cl \cdot H_2O$) C, H, N.

1-Acetoxy-6-bromo-6-desmethyl-*N*-methylmitosene (14). A solution of **12** (220 mg, 0.6 mmol) in 75 mL of acetic acid containing 6.6 g of sodium acetate trihydrate was treated with 3.5 mL of a solution containing 0.195 mol of bromine/mL of acetic acid. The mixture was stirred under N_2 in the dark for 2 h, treated with another 1 mL of bromine solution, and stirred 2 h more and treated with ice water and methylene chloride. The organic layer was washed with $NaHCO_3$ solution until gas evolution ceased, dried over Na_2SO_4, and concentrated to give 159 mg (60%) of **14**. An analytical sample, purified by preparative chromatography on a silica gel plate with methylene chloride containing 5% acetone as solvent followed by crystallization from acetone had mp 125 °C dec; NMR δ ($CDCl_3$) 6.35 (dd, 1 H), 5.32 (d, 2 H), 4.35 (m, 2 H), 4.2 (s, 3 H), 2.8 (d, 3 H), 2.7 (m, 2 H), 2.1 (s, 3 H), 2.0 (s, 3 H for acetone); IR 3400, 1740–1720, 1655 cm^{-1}; MS, m/z (M – CH_3NCO) 383, 385. Anal. ($C_{17}H_{17}BrN_2O_7 \cdot 0.5C_3H_6O$) C, H, N.

1-Acetoxy-7-methoxy-*N*-methylmitosene (16). A solution of 1-acetoxy-7-methoxydecarbamoylmitosene[8] (180 mg, 0.52 mmol) in 50 mL of methylene chloride was treated with methyl isocyanate (1 mL, excess) and triethylamine (1 mL). The mixture was stirred under N_2 overnight and then treated with 100 mL of ice water. The organic layer was dried over Na_2SO_4 and concentrated to yield an oil, which was purified by preparative chromatography on a silica gel plate with methylene chloride containing 5% acetone as solvent. Concentration of the main yellow band gave 45.6 mg (23%) of **16** as a yellow solid: mp 173 °C dec; NMR ($CDCl_3$) δ 6.3 (dd, 1 H), 5.3 (s, 2 H), 4.9 (s, 1 H), 4.3 (t, 2 H), 4.0 (s, 3 H), 2.75 (d, 3 H), 2.6 (m, 2 H), 3.1 (s, 3 H), 2.9 (s, 3 H); IR 3340, 1735, 1700, 1660, 1645 cm^{-1}; MS, m/z (M) 376. Anal. ($C_{18}H_{20}N_2O_7 \cdot 0.5H_2O$) C, N, H: calcd, 5.76; found, 5.17.

1-Acetoxy-6-desmethyl-7-pyrrolidino-*N*-methylmitosene (17). A mixture of **12** (59 mg, 0.16 mmol), cupric acetate trihydrate (147 mg), and 200 mL of methanol was stirred and saturated with oxygen gas. Pyrrolidine (0.2 mL, 2.39 mmol) was added and the mixture was stirred 1 h in the dark. It was filtered and concentrated and the residue was purified by preparative-layer chromatography on silica gel with methylene chloride containing 20% acetone as solvent. Two main purple bands appeared. The band with higher R_f gave 36 mg (56%) of **17** as a purple solid: mp 194 °C dec; NMR ($CDCl_3$) δ 6.3 (dd, 1 H), 5.32 (s, 2 H), 5.3 (s, 1 H), 5.1 (s, 1 H), 4.3 (m, 2 H), 3.6 (br s, 4 H), 2.8 (d, 3 H), 2.7 (m, 2 H), 2.1 (s, 3 H), 1.9 (br s, 4 H); IR 3390, 1720, 1660 cm^{-1}; MS, m/z (M) 401. Anal. ($C_{20}H_{23}N_3O_6$) C, H, N.

1-Acetoxy-7-amino-6-bromo-6-desmethyl-*N*-methylmitosene (18). A stirred solution of **12** (84 mg, 0.19 mmol) in 20 mL of methylene chloride was cooled in a dry ice–ethanol bath and ammonia was bubbled in until the solution turned purple (10 min). The resulting solution was concentrated and the residue was purified by chromatography on silica gel with methylene chloride containing 5% acetone as solvent. Crystallization of the product from acetone gave 36 mg (44%) of **18** as purple solid: mp 152 °C; NMR ($CDCl_3$) δ 6.3 (dd, 1 H), 5.65 (s, 1 H), 4.35 (m, 2 H), 2.8 (d, 3 H), 2.7 (m, 2 H), 2.1 (s, 3 H + 6 H from acetone); IR 3390, 1720, 1660 cm^{-1}; MS, m/z 427, 425. Anal. ($C_{16}H_{16}BrN_3O_6 \cdot C_3H_6O$) C, H, N.

Ethyl 2,3-Dihydro-7-methoxy-1*H*-pyrrolo[1,2-*a*]indole-9-carboxylate (21). A mixture of **19** (1.76 g, 11.4 mmol),[19] dry methanol (70 mL), acetic acid (5.6 mL), and freshly sublimed 1,4-benzoquinone (2.46 g, 22.8 mmol) was stirred for 48 h. The crude product (**20**, 740 mg) was collected by filtration and washed with methanol and methylene chloride. It was used directly in the next step.

A mixture of crude **20** (70 mg), dimethyl sulfate (0.6 mL), potassium carbonate (2.0 g), and acetone (27 mL) was stirred under N_2 at reflux temperature overnight. It was filtered and the filtrate was concentrated to give 74 mg (corresponding to 50% overall yield) of **21** as white solid: mp 120 °C; NMR ($CDCl_3$) δ

7.6 (dd, 1 H), 7.1 (d, 1 H), 6.75 (dd, 1 H), 4.4 (q, 2 H), 4.0 (dd, 2 H), 3.9 (s, 3 H), 3.3 (dd, 2 H), 2.7 (m, 2 H), 1.45 (t, 3 H); IR 2940, 1670 cm^{-1}. Anal. ($C_{15}H_{17}NO_3 \cdot 0.2H_2O$) C, H, N.

Ethyl 1-Acetoxy-2,3-dihydro-7-methoxy-8-nitro-1*H*-pyrrolo[1,2-*a*]indole-9-carboxylate (25). A mixture of **21** (646 mg, 2.5 mmol), freshly prepared lead tetraacetate (2.43 g, 4.4 mmol),[20] and acetic acid (60 mL) was stirred under N_2 for 10 h. Water was added and the mixture was filtered and extracted with ether. The organic layer was washed with $NaHCO_3$ solution and brine, dried over $MgSO_4$, and concentrated. The oily residue was chromatographed on silica gel with chloroform–ethyl acetate (4:1) as solvent. This procedure gave 91 mg of **24**, which was used directly in the next step.

A mixture of **24** (60 mg), acetic acid (3 mL), and concentrated nitric acid (0.1 mL) was stirred for 1 h, cooled in an ice bath, and treated with 20 mL of ice water. The precipitate that formed was dissolved in 80 mL of methylene chloride and this solution was washed with brine, dried over $MgSO_4$, and concentrated. This procedure gave 66 mg (7% overall) of **25** as yellow solid: mp 169–173 °C; NMR ($CDCl_3$) δ 7.4 (dd, 1 H), 7.1 (dd, 1 H), 6.5 (dd, 1 H), 4.3 (m, 4 H), 3.95 (s, 3 H), 2.9 (m, 2 H), 2.1 (s, 3 H), 1.3 (t, 3 H); IR 1740, 1700, 153, 1370 cm^{-1}. Anal. ($C_{17}H_{18}N_2O_7 \cdot 0.05CH_2Cl_2$) C, H, N.

Ethyl 1-Acetoxy-2,3-dihydro-5,8-dioxo-7-methoxy-1*H*-pyrrolo[1,2-*a*]indole-9-carboxylate (22). A mixture of **25** (160 mg, 0.44 mmol), tin (630 mg, excess), ethanol (60 mL), and 3 N HCl (15 mL) was stirred for 3 h and then treated with dilute $NaHCO_3$ solution. The resulting mixture was extracted with chloroform and this extract was washed with brine, dried over Na_2SO_4, and concentrated. The oily residue (160 mg) containing **26** was used directly in the next step.

A solution of crude **26** in 60 mL of acetone was treated with a solution of potassium nitrosodisulfonate (1.34 g) in 85 mL of pH 6 potassium dihydrogen phosphate. After 4 h, water and methylene chloride were added. The organic layer was washed with 5% Na_2CO_3 solution, dried over $MgSO_4$, and concentrated. Purification of the residue by chromatography on silica gel with methylene chloride containing 5% acetone as solvent gave 78 mg (51%) of **22** as yellow solid: mp 81 °C; NMR ($CDCl_3$) δ 6.3 (dd, 1 H), 5.7 (s, 1 H), 4.4 (m, 4 H), 3.9 (s, 3 H), 2.7 (m, 2 H), 2.1 (s, 3 H), 1.3 (t, 3 H); IR 1730, 1720, 1635 cm^{-1}. Anal. ($C_{17}H_{17}NO_7 \cdot 0.1CH_2Cl_2$) C, H, N.

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Registry No. 1, 4792-58-9; **2**, 96000-33-8; **3**, 96000-34-9; **4**, 96000-35-0; **5**, 96000-36-1; **5** (phenylcarbonate ester), 96000-53-2; **6**, 96000-37-2; **7**, 96000-38-3; **8**, 96000-39-4; **9**, 96000-40-7; **10**, 96000-41-8; **11**, 96000-42-9; **12**, 96000-43-0; **13**, 96000-44-1; **14**, 96000-45-2; **16**, 96000-46-3; **17**, 96000-47-4; **18**, 96000-48-5; **19**, 35150-22-2; **20**, 31676-32-1; **21**, 71948-62-4; **22**, 96000-49-6; **24**, 96000-50-9; **25**, 96000-51-0; **26**, 96000-52-1; 1-acetoxy-7-methoxydecarbamoylmitosene, 40863-76-1; methyl isocyanate, 624-83-9.

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Synthesis and Antiherpetic Activity of (*S*)-, (*R*)-, and (±)-9-[(2,3-Dihydroxy-1-propoxy)methyl]guanine, Linear Isomers of 2′-Nor-2′-deoxyguanosine[1]

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Racemic 9-[(2,3-dihydroxy-1-propoxy)methyl]guanine [(±)-iNDG], a new analogue of acyclovir (ACV) and a structural analogue of 2′-nor-2′-deoxyguanosine (2′NDG), was synthesized and found to inhibit the replication of herpes simplex virus types 1 (HSV-1) and 2 (HSV-2). Subsequently, its optical isomers, (*R*)- and (*S*)-iNDG, were prepared from chiral intermediates. The chloromethyl ethers of 1,2-di-*O*-benzyl-D- and -L-glycerol were made and reacted with tris(trimethylsilyl)guanine to give the 9-alkylated guanines, which were deprotected by catalytic hydrogenolysis. Against HSV-1 and HSV-2 in cell culture, (*S*)-iNDG was approximately 10- to 25-fold more active than the *R* enantiomer and had an ED_{50} comparable to those for ACV and 2′NDG. The inferior activity of (*R*)-iNDG paralleled the poor inhibition of viral DNA polymerase by its phosphorylation products. In mice infected intraperitoneally or orofacially with HSV-1 or intravaginally with HSV-2, (*S*)-9-[(2,3-dihydroxy-1-propoxy)methyl]guanine [(*S*)-iNDG] was less efficacious than 2′NDG but comparable to or more active than ACV.

One of the most promising approaches to the chemotherapy of herpes simplex virus (HSV) infections has been to utilize the broad specificity of the HSV-coded thymidine kinase to generate antiviral phosphorylated nucleosides or nucleoside analogues in infected cells. Thus, the high therapeutic ratio of acyclovir (ACV, **1**), an acyclic analogue of 2′-deoxyguanosine (**2**), is attributed to selective phosphorylation in infected cells by the HSV thymidine kinase and further phosphorylation by host cell enzymes to the triphosphate, a potent inhibitor of the viral DNA polymerase.[2]

In order to more closely mimic the structure of 2′-deoxyguanosine, we[3] and, independently, others[4-6] synthesized an analogue, 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (**3**) or 2′-nor-2′-deoxyguanosine (2′NDG;

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