elucidated exactly will be the subject of future studies.

Edo et al. reported that Trp83 must exist at the binding site because of its resistance against oxidation in the chromophore-bound structure.[11,12] However, in the model, Trp83 is located far from the binding site and contributes to the formation of the hydrophobic core in the small unit, whereas Trp39 is close to the binding site of the carbonate group of the chromophore. Therefore, it is not unlikely that upon binding of the chromophore the protein structure becomes more rigid and so does the side chain of Trp83, which is kept buried inside. This also suggests a rigid conformation of the chromophore at the binding cleft.

Conclusion

The significant increase of the thermal, chemical, and photochemical stability of the chromophore in the complexed form[8,9] is likely a result of the following interactions: (1) the hydrophobic environment of the epoxide and the unsaturated bonds; (2) steric coverage of the active reaction site (C12) with Phe78 and the methyl group (C6') of the amino sugar moiety; (3) stabilization of a rigid conformation of the chromophore by a network of hydrogen bonds and hydrophobic interactions at the binding cleft; and (4) more interestingly, the possible interaction between the sulfur atom of Cys37 and the C2–C3 acetylenic bond. While the last interaction awaits experimental verification, the close intermolecular contact reminds us of the intramolecular trisulfide bond of the structurally related potent antitumor antibiotics calicheamicins[33] and esperamicins.[34] A possible stabilizing interaction of the trisulfide or disulfide unit is experimentally and theoretically under investigation in these laboratories.

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Registry No. Neocarnostatin apoprotein, 101359-79-9; neocarzinostatin chromophore, 81604-85-5.

Supplementary Material Available: Tables of atomic coordinates for the NCS chromophore and for Cα of apo-NCS (4 pages). Ordering information is given on any current masthead page.

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Synthesis and Antitumor Evaluations of Symmetrically and Unsymmetrically Substituted 1,4-Bis[(aminoalkyl)amino]anthracene-9,10-diones and 1,4-Bis[(aminoalkyl)amino]-5,8-dihydroxyanthracene-9,10-diones

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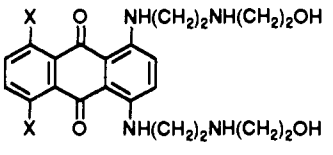
The ipso bis displacements of fluoride from 1,4-difluoroanthracene-9,10-dione (3) and 1,4-difluoro-5,8-dihydroxyanthracene-9,10-dione (4) by excess of a diamine (or a monoamine) in pyridine at room temperature lead to the symmetrically substituted 1,4-bis-substituted analogues 5 and 6, respectively. The ipso monodisplacements of fluoride from 3 and 4 can be accomplished by treatment with less than 1 molar equiv of a diamine (or a monoamine) to yield 7 and 8, respectively. Treatment of 7 or 8 with a different diamine leads to the unsymmetrically substituted 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones 9 and 10, respectively. Many of the synthetic unsymmetrical analogues have been evaluated for their antitumor activity against L1210 in vitro and in vivo. Cross resistance of analogue 10a with mitoxantrone (2) and doxorubicin was evaluated against MDR lines in vitro against human colon carcinoma LOVO and its subline resistant to DOXO (LOVO/DOXO). Potential mechanisms for the observed cytotoxicity are presented and discussed.

The discovery of the antitumor activity of 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones such as ametantrone (1) and mitoxantrone (2)[1-4] has led to numerous physicochemical and pharmacological studies on the tumoricidal mechanisms of these chemotypes.[5]



1, X = H
2, X = OH

Mitoxantrone (2) is an important new drug with demonstrated clinical efficacy in the treatment of leukemia,

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lymphomas, and breast cancer and has FDA approval for the treatment of acute nonlymphocytic leukemia.[6] While the diones 1 and 2 have a narrower spectra of anticancer activity in comparison with the anthracyclines such as DOXO, this compound is quite cardiotoxic while 1 and 2 are significantly less cardiotoxic.[6,7] However, the need exists for the development of anthracenedione congeners with improved therapeutic indices and effectiveness against MDR cell lines.[8]

Although the mechanism(s) of action of the antitumor activity of the anthracene-9,10-diones 1 and 2 is probably multimodal in nature, a number of studies have indicated that an intercalative interaction with DNA may be a major cellular event.[9] Although this may be an important event, it, in itself, is not sufficient to rationalize the antitumor activity of these chemotypes. Nonetheless, intercalation may serve as a mechanism to "anchor" the drug at specific base pair sites of the DNA until a critical cell killing event occurs. Evidence has also been presented that the pharmacological effects of 1 and 2 might involve nucleic acid condensations (of RNA in nucleoli).[9e-g]

Biophysical and biochemical studies have led to a reasonably clear picture of the structure of the DNA–anthracenedione intercalation complexes.[5] Techniques such as electron microscopy,[10] high-field NMR spectroscopy,[11] modern computer graphics,[12] and ab initio calculations[13] have provided valuable data on the nature of these intercalation complexes. The importance of the structure of the side arms for maximal antitumor activity is indicated in the choice of 1 and 2 (from several hundred analogues) for clinical use.[1,2]

DNA topoisomerases have also surfaced as potential targets involved in the cell killing mechanism. It has been proposed that a drug–DNA-topoisomerase ternary complex may be responsible for the DNA cleavages induced by intercalative drugs such as 2.[14] The antitumor activity of molecules such as 1 and 2 could also be partially due to the free radical intermediates formed during reductive (anion-radicals) or oxidative (anion-cations) metabolic activation. The anion-radical can enter the redox cycle with molecular oxygen leading to destructive oxygen radicals.[15]

Evidence suggests that redox cycling in the anthracyclines may be more involved in cardiotoxic (2 is relatively noncardiotoxic) rather than cytotoxic effects.[16] The formation of free radicals in liver microsomes from several cytotoxic (alkylamino)anthracenediones has been detected by ESR, and superoxide radicals were generated in the presence of oxygen.[17] On the other hand, numerous studies of 1 and 2 do not support metabolic stimulation of oxygen uptake or lipid peroxidation.[18-22]

Attempts to establish a relationship between intercalative binding (quantified as a binding affinity constant) and antitumor activity have been reported and their success is dependent on the particular chromophore.[9a] In general, anticancer efficacy does not correlate with DNA binding affinity. Drug–DNA binding constants for 1 and 2 and related congeners with calf thymus DNA show a large sensitivity of the binding constant to the presence of the OH substitution at the 5,8 positions and the nature of the side chains.[23-29]

The dissociation rate constant for the DNA–ligand complex has also been used as a probe to assess anticancer activity. The rationale for this being that the side arms could influence the dissociation rate of the ligand–DNA complex and increase the time the ligand occupies a particular binding site.[30,31]

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The goal of the present study was an evaluation of the importance of the side-arm substitution patterns at the 1 and 4 positions of the anthracene-9,10-dione skeleton: not only would the nature of the side arms influence the binding or kinetic dissociation rate of the anthracenedione–DNA intercalant but also they might be intimately involved in interaction with the topoisomerase II enzyme.[32]

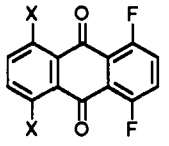
We report a convenient synthetic pathway that leads to symmetrically or unsymmetrically substituted 1,4-bis(aminoalkyl)aminoanthracene-9,10-diones. The biological evaluations of the antitumor activities of the unsymmetrical analogues have been performed and the results will be discussed.

Chemistry

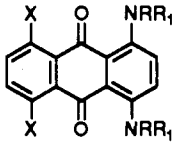
The usual methodology for the synthesis of congeners related to **1** and **2** is by treatment of leucoquinizarin or 5,8-dihydroxyleucoquinizarin, respectively, with the appropriate diamine followed by oxidation (usually air during workup).[2] During the course of our research, it was reported that the synthesis of unsymmetrically substituted analogues related to **1** could be effected by sequential treatment of leucoquinizarin with a limited amount of one diamine followed by addition of a second diamine and oxidative workup.[4] A route involving side-arm buildup has also led to a few unsymmetrical analogues related to **1**. Treatment of quinizarin with diamines also leads to analogues of **1**.[2]

Other procedures of some synthetic generality are based on ipso substitutions of various leaving groups at the 1,4 positions of the anthracene-9,10-dione by nitrogen nucleophiles. Substitutions of anthracene-9,10-diones having 1,4-ditosylate,[33,34] 1,4-ditriflate,[33], 1,4-dinitro,[35] 1,4-dichloro,[36] and 1,4-dimethoxy[37] substituents by nitrogen nucleophiles have been reported. Usually the displacement of the first group is relatively easy but the displacement of the second group (deactivated by the nitrogen substitution) can lead to problems of dealkylation[33] and cyclization side products[37] since higher temperatures are required for its substitution.

We have found that 1,4-difluoroanthracene-9,10-dione (**3**)[38] and 1,4-difluoro-5,8-dihydroxyanthracene-9,10-dione (**4**)[39] undergo facile ipso substitutions of the fluoride groups

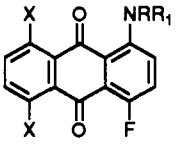


3, X = H
4, X = OH

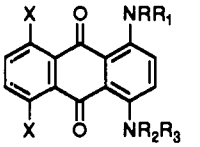


5, X = H
6, X = OH

by diamines at room temperature in pyridine and lead to the 1,4-bis-substitution products **5** and **6**, respectively. The mono-substituted products **7** and **8** can be isolated and treated with a different diamine to yield 1,4-bis unsymmetrically substituted analogues **9** and **10**, respectively, in good yields.



7, X = H
8, X = OH



9, X = H
10, X = OH

Treatment of **3** or **4**, respectively, with the appropriate amines in DMSO or pyridine as solvent (room temperature) led to the bis-substitution products **5** and **6**, respectively, which are tabulated in Table I.

The data in Table I indicate that the stepwise ipso substitution of the fluorides of **3** or **4** is a useful preparative route for 1,4-bis(alkylamino)- and 1,4-bis[(aminoalkyl)amino]anthracene-9,10-diones related to **5** and **6**. Of additional note is the preparation of the *N*-CH_3 substitution product **5f**, which differs from the numerous prior analogues[1,2] in lacking the potential for H-bonding with the adjacent C=O groups.

The synthesis of mitoxantrone (**2**) by treatment of **4** with 2-[(2-aminoethyl)amino]ethanol was successful; however, competitive formation of considerable amounts of the tetrahydroquinoxaline **11** occurred. The OH groups activate the ring for a Michael-type cyclization, which ultimately leads to **11**.

Treatment of **3** or **4** with a limited amount of the amine in pyridine, DMSO, or chloroform (at room temperature) led to the mono-substituted anthracene-9,10-diones **7** or

Table I. 1,4-Bis[amino-substituted]anthracene-9,10-diones **5** and **6** Prepared by Ipso Substitutions by Amines or Diamines on **3** or **4**

compd	X	R	R_1	% yield
5a	H	H	C_6H_5	75
5b	H	H		88
5c	H	H	$(CH_2)_2OH$	93
5d	H	H	$(CH_2)_2NH_2$	60
5e	H	H	$(CH_2)_2N(CH_3)_2$	86
5f	H	CH_3	$(CH_2)_2N(CH_3)_2$	80
5g	H	H		10
6a (=1)	H	H	$(CH_2)_2NH(CH_2)_2OH$	30
6b (=2)	OH	H	$(CH_2)_2NH(CH_2)_2OH$	30[a]
6c	OH	H	$(CH_2)_2N(CH_3)_2$	87

[a] Substantial amounts of the cyclized product **11** were also formed and chromatographic purification of **6b** was tedious.

Table II. 1-[Amino-substituted]-4-fluoroanthracene-9,10-diones **7** and **8** Prepared by Mono-substitutions by Amines or Diamines on **3** and **4**

compd	X	R	R_1	% yield
7a	H	H	$(CH_2)_2N(CH_3)_2$	47
7b	H	H	$(CH_2)_2NH_2$	33
7c	H	CH_3	$(CH_2)_2N(CH_3)_2$	67
7d	H	H	$(CH_2)_2OH$	50
7e	H		$-(CH_2)_2-$	19
7f	H	H		24
8	OH	H	$(CH_2)_2N(CH_3)_2$	42

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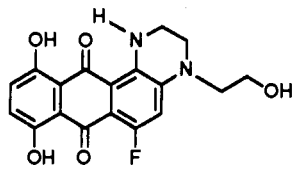

11

8, respectively. The compounds prepared by this route are tabulated in Table II.

One notes from the data of Table II that good yields of the mono-fluoro-substituted analogues can be obtained.

The mono-substituted analogues **7** or **8** could then be treated with a different diamine in pyridine or DMSO as solvent (room temperature) to lead to the unsymmetrical compounds **9** or **10**, respectively, which are tabulated in Table III.

The congeners prepared in Table III indicate that this synthetic methodology is extremely flexible in the preparation of unsymmetrical analogues in good yields. Clearly the procedure could be adapted to the preparation of additional new analogues.

Biological Studies

Many of the analogues that we prepared were evaluated as inhibitors of the growth of L1210 cells in vitro and in vivo. These data are tabulated in Table IV.

The effectiveness of the most active analogue **10a** was further evaluated against MDR cell lines in vitro and in vivo. The cytotoxicity of **10a** in comparison with amentantrone (**1**), mitoxantrone (**2**), and DOXO was evaluated against two human colon carcinoma lines sensitive and resistant to DOXO (LOVO and LOVO/DOXO), and the results are tabulated in Table V.

Discussion

The inherent goal of this research was an exploration of the effect on biological activity of the substitution of anthracenedione chemotypes related to **1** and **2** but bearing differing (aminoalkyl)amino side arms at the 1 and 4 positions. Our prior investigation was the study of several unsymmetrically substituted analogues (such as **9c**) that showed excellent in vitro activity but were inactive in vivo.[3]

The difluoroanthracene-9,10-dione **3** and the monosubstituted fluoro analogue **7a** showed in vitro values of 5.6 and 1.15 μg/mL, respectively, and were not evaluated in vivo. The bis-substituted cyclopropylamine analogue **5b**, as anticipated, was found to be inactive. It is of interest to note the effect of biological activity on the substitution of both hydrogens of the vinylogous NH groups present in **5e** by methyl groups to give **5f**. Compound **5e** is quite active in vitro [ID_{50}(L1210) = 0.088 μg/mL] and exhibits a %T/C = 150 at a dosage of 50 mg/kg (QD 1–9)][1c] while **5f** is 52 times less active in vitro and exhibits no in vivo activity. The replacement of one hydrogen of the NH groups of **5e** by a methyl group to give **9d** leads to much less significant decrease in biological activity. Compound **9d** is less active by a factor of 5 in the in vitro comparison with **5e** and is moderately active in the in vivo screen.

The side arms of **5e** are held in positions that will not impede intercalation by the strong hydrogen bonding between the adjacent NH and C=O groups. On the other hand, the side arms of **5f** would be expected to be forced out of the plane defined by the rings and intercalation would be more difficult. The activity of **9d**, on the other hand, is still partially maintained even though only one side arm can hydrogen bond.

The unsymmetrically substituted analogues **9a** and **9b** exhibit excellent in vitro and in vivo activities. Compound **9b** has also been shown to have excellent activity in an in vivo P388 murine leukemia screen.[4a] The introduction of 5,8-dihydroxyl substitution into **9b** to give **10a** substantially increases the activity and indeed this activity of **10a** is comparable to that of mitoxantrone (**2**). The analogues **9c** and **10b**, which have one side arm with four methylene groups holding the terminal amino group, show substantially diminished activity.

Table III. Unsymmetrical 1,4-Bis[(aminoalkyl)amino]anthracene-9,10-diones **9** and **10** Prepared from Monofluoro Analogues **7** and **8**

compd	X	R	R_1	R_2	R_3	% yield
9a	H	H	$(CH_2)_2NH_2$	H	$(CH_2)_2N(CH_3)_2$	87
9b	H	H	$(CH_2)_2NH(CH_2)_2OH$	H	$(CH_2)_2N(CH_3)_2$	94
9c	H	H	$(CH_2)_4NH_2$	H	$(CH_2)_2N(CH_3)_2$	90
9d	H	CH_3	$(CH_2)_2N(CH_3)_2$	H	$(CH_2)_2N(CH_3)_2$	45
10a	OH	H	$(CH_2)_2NH(CH_2)_2OH$	H	$(CH_2)_2N(CH_3)_2$	48
10b	OH	H	$(CH_2)_4NH_2$	H	$(CH_2)_2NH(CH_2)_2$-OH	59

The most active analogue **10a** in the L1210 test system was more cytotoxic than **2** and DOXO in both the sensitive and resistant (MDR) human colon carcinoma sublines LOVO and LOVO/DOXO. Compound **10a** is only partially cross resistant with DOXO, showing a significantly lower resistance index than **1** or **2** (Table V). The higher in vivo potency of **10a** with respect to **1** and **2** is also shown against P388 murine leukemia [**10a**, ID_{50} = 0.1 ng/mL; **1**, ID_{50} = 27.0 ng/mL; **2**, ID_{50} = 0.43 ng/mL].

It has been suggested that the side arms present in the anthrapyrazoles (and **1** and **2**) may be "associated in part with an optimal range of hydrophobic–lipophilic balance".[40] In a recent study we have prepared the analogue of **1** where the distal side arm H of the hydroxyl group has been changed to a methyl group. This latter compound was found to be about 50 times less potent in a L1210 in vitro assay.[41] The importance of the OH group is indicated by this result. A tetrahydrobenz[*a*]anthraquinone analogue of **2** has recently been reported to have modest antitumor activity.[42]

The rationalization of the antitumor activities exhibited in vivo via redox cycling as reflected in half-wave reduction potentials does not appear very likely. Congeners such as **9b** and **9c** (or **10a** and **10b**) would have almost identical half-wave potentials since the side arms would have little effect on this property.[43,44]

The DNA binding constants for **2** and analogue **6** [where R = H, R_1 = $(CH_2)_4N(CH_3)_2$] have been reported to be similar and only **2** shows antileukemic activity.[25] Mitoxantrone (**2**) and its homologue **6** [where R = H, R_1 = $NH(CH_2)_3NH(CH_2)_2OH$] have binding constants with calf thymus DNA 17.8 × 10^4 [%T/C(P388) = 299 at 0.4 mg/kg] and 23.5 × 10^4 [%T/C(P388) = 291 at 50 mg/kg], respectively, which is a reverse correlation for the binding constant and the antitumor activity (**2** is 100 times as potent as **6** on a dosage basis).[23]

The dissociation rates of the DNA complexes of **2**, **1**, and **5e** are 0.82, 2.7, and 2.9 s^{-1}, respectively, at least a trend

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Table IV. Activities of Analogues Against L1210 in Vitro and in Vivo

compd[a]	ID_{50} (μg/mL)	dose, mg/kg	treat. sched[b]	%T/C[c]	LTS[d]	TOX[e]
5b	>10	25	1, 5, 9	107	0/6	0/6
		12.5	1, 5, 9	112	0/6	0/6
		6.25	1, 5, 9	100	0/6	0/6
5f	4.6	50	1	95	0/6	0/6
		25	1	100	0/6	0/6
		12.5	1	103	0/6	0/6
6a (=1)	<0.01	50	1, 5, 9	146	0/6	0/6
		25	1, 5, 9	252	1/6	0/6
		12.5	1, 5, 9	177	0/6	0/6
9a	0.03	50	1, 5, 9	180	0/6	0/6
		25	1, 5, 9	150	0/6	0/6
		12.5	1, 5, 9	129	0/6	0/6
9b	0.0038	50	1, 5, 9	145	0/6	0/6
		25	1, 5, 9	148	0/6	0/6
9c	0.0045	50	1, 5	64	0/6	4/6
		25	1, 5	111	0/6	2/6
		12.5	1, 5, 9	113	0/6	0/6
9d	0.5	50	1, 5, 9	127	0/6	0/6
		25	1, 5	108	0/6	0/6
10a	0.00017	25	1, 5, 9	244	0/6	0/6
		12.5	1, 5, 9	187	0/6	0/6
		6.25	1, 5, 9	188	0/6	0/6
10b[f]	0.0007	5	1, 5	138	0/6	0/6

[a] See Tables I and III for structures. [b] CDF1 mice were injected ip with 10^6 cells/mouse; treatment was given ip on day 1, days 1, 5, or days 1, 5, 9 after tumor transplantation (day 0). [c] Mean survival time of treated mice/mean survival time of controls × 100. [d] Long-term survivors at the end of the experiment (60 days). [e] Number of toxic deaths/total number of mice. [f] Limited amount of sample precluded further examination.

Table V. In Vitro Cytotoxic Activity of **10a** in Comparison to Mitoxantrone, Ametantrone, and Adriamycin

	IC_{50} (ng/mL)[a]		
	LOVO[b]	LOVO/DOXO[b]	R.I.[c]
10a	1.0	24.0	24.0
mitoxantrone (2)	5.5 ± 0.5	274.9 ± 28.4	50.2 ± 5.4
ametantrone (1)	282.5 ± 44.8	44765 ± 22056	169 ± 57.8
adriamycin	24.7 ± 6.2	3841 ± 342	148.7 ± 31.4

[a] Inhibiting concentration 50% of cellular growth. [b] Tumor cell lines: LOVO - LOVO/DOXO (human colon carcinoma). [c] IC_{50} resistant line/IC_{50} sensitive line.

in the direction of decreased cytotoxicity.[30b]

It might be speculated that the antitumor activities expressed by the anthracenediones of the type studied here are based on two molecular portions of the molecules. The anthracenedione skeleton acts as the DNA intercalant and the side arms at positions 1 and 4 interact with the topoisomerase II enzyme.[32]

We hope to perform pharmacokinetic and pharmacodynamic studies on several of the active unsymmetrical analogues to shed more light on their mechanism of cell kill. Studies with topoisomerase II will also be performed.

Conclusions

A synthetic strategy that leads to symmetrically or unsymmetrically substituted 1,4-bis[(aminoalkyl)amino]-anthracene-9,10-diones have been developed.

Unsymmetrically substituted 1,4-bis[(aminoalkyl)-amino]anthracene-9,10-diones have been shown to have high antitumor activities. The presence of one side arm similar to the ones of **1** and **2** is sufficient to lead to very effective compounds. The level of activity, however, is further modulated by the nature of the second side arm.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Proton and ^{13}C NMR were run on a Bruker WP-270SY or WM-250 pulsed Fourier transform spectrometer. Precoated silica gel and alumina plates (Eastman Chromagram sheets) with fluorescent indicator were used for thin layer chromatography (TLC). Baker analyzed 80–200-mesh silica gel was used for column chromatography. Mass spectra were run on a Finnigan MAT 4610 spectrometer. Microanalyses were performed by Robertson Laboratory, Madison, NJ.

1,4-Bis(phenylamino)anthracene-9,10-dione (5a). Freshly distilled aniline (80 mg, 0.86 mmol) was added to **3** (50 mg, 0.20 mmol) in DMSO (0.75 mL), and the mixture was heated in an oil bath held at 80 °C with stirring for 24 h. On cooling, the mixture was added to water (50 mL), and the solid was collected by filtration. Recrystallization from petroleum ether/ethanol yielded **5a** (60 mg, 75%): mp 220–221 °C [lit.[45] mp 217 °C]; ^1H NMR ($CDCl_3$) 12.25 (s, 2 H), 8.39 (m, 2 H), 7.76 (m, 4 H), 7.50 (s, 2 H), 7.39 (m, 4 H), 7.28 (m, 2 H).

1,4-Bis(cyclopropylamino)anthracene-9,10-dione (5b). Cyclopropylamine (288 mg, 5.0 mmol) was added to **3** (118 mg, 0.48 mmol) in DMSO (2 mL), and the red solution that quickly formed was stirred at room temperature for 72 h. The mixture was quenched into ice–water (100 mL), and the solid was collected by filtration and dried (126 mg, 88%). TLC analysis (30% pentane–70% chloroform) indicated a trace amount of the red mono-substitution product. Recrystallization from ligroin gave blue needles of mp 263–266 °C: ^1H NMR ($CDCl_3$) 10.6 (s, 2 H), 8.3 (m, 2 H), 7.75 (s, 2 H), 7.70 (m, 2 H), 2.7 (m, 2 H), 0.95 (m, 4 H), 0.75 (m, 4 H); mass spectrum, *m/z* (relative intensity) 318 M^+ (100). Anal. ($C_{20}H_{18}N_2O_2$) C, H, N.

1,4-Bis[(2-hydroxyethyl)amino]anthracene-9,10-dione (5c). Dione **3** (101 mg, 0.43 mmol) and 2-aminoethanol (287 mg, 4.70 mmol) in DMSO (1 mL) were stirred at room temperature for 48 h. The mixture was quenched into a cold saturated NaCl solution, and this was allowed to stand overnight. The blue solid was collected by filtration and dried (130 mg, 93%). Analysis by TLC (silica gel, 5% methanol/95% chloroform) showed a single blue spot. The compound could be crystallized from methanol to give a compound with mp 236–237 °C [lit.[46] mp 242.5–244.0 °C]: ^1H NMR (DMSO-d_6) 10.98 (t, 2 H), 8.28 (m, 2 H), 7.70 (m, 2 H), 7.38 (s, 2 H), 4.91 (t, 2 H), 3.74 (m, 4 H), 3.55 (m, 4 H); mass spectrum, *m/z* (relative intensity) 326 M^+ (51), 298 (100), 264 (10).

1,4-Bis[(2-aminoethyl)amino]anthracene-9,10-dione (5d). A mixture of **3** (240 mg, 1.0 mmol) and 1,2-diaminoethane (240 mg, 4 mmol) in pyridine (2 mL) was stirred at room temperature for 79 h. Pentane (10 mL) was added to the mixture and the blue precipitate was collected by filtration. TLC (silica gel, $CHCl_3$/MeOH 4:1) showed the presence of a major blue spot. The crude solid was heated in $CHCl_3$ and filtered from some insoluble

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material, and pentane was added to the filtrate. On cooling, **5d** (180 mg, 60%) was obtained: mp 165–166 °C [lit,[2a] mp 174–176 °C]; ^{1}H NMR ($CDCl_3$) 10.85 (b, 2 H), 8.37 (m, 2 H), 7.70 (m, 2 H), 7.25 (s, 2 H), 3.4 (m, 4 H), 3.09 (m, 4 H), 1.4 (b, undefined integral, NH_2 coalesced with water): mass spectrum, m/z (relative intensity) 334 M^+ (100), 294 (15), 277 (40), 256 (5).

1,4-Bis[[(2-dimethylamino)ethyl]amino]anthracene-9,10-dione (5e). A mixture of **3** (104 mg, 0.43 mmol) and *N,N*-dimethylethylenediamine (394 mg, 4.5 mmol) in DMSO (1 mL) was stirred at room temperature for 44 h and then quenched into a cold saturated NaCl solution (100 mL). The mixture on standing overnight was filtered to yield **5e** (140 mg, 86%). Analysis of this crude product by TLC indicates one dominant blue spot (50% CH_3OH/50% $CHCl_3$). The solid was recrystallized from $CHCl_3$/petroleum ether: mp 173–174 °C [lit.[2b] mp 172–173 °C]; ^{1}H NMR 10.75 (b, 2 H), 8.38 (m, 2 H), 7.7 (m, 2 H), 7.22 (s, 2 H), 3.52 (m, 4 H), 2.68 (t, 4 H), 2.38 (s, 12 H).

1,4-Bis[methyl[2-(dimethylamino)ethyl]amino]-anthracene-9,10-dione (5f). A mixture of **3** (100 mg, 0.41 mmol) and *N,N,N'*-trimethylethylenediamine (486 mg, 4.76 mmol) in DMSO (1 mL) was allowed to stir at room temperature for 48 h. The deep blue reaction mixture was quenched into a cold saturated NaCl solution (80 mL) upon which a blue oil separated, which adhered to the walls of the beaker. The mixture was extracted with CH_2Cl_2, and the extracts were dried over sodium sulfate and concentrated on the rotary evaporator to yield a deep bluish oily product (134 mg, 80%). Analysis by TLC (silica gel, 80% $CHCl_3$/20% CH_3OH with 1 drop of Et_3N) showed a single blue spot. Crystallization attempts from a number of solvents were unsuccessful and preparation of an analytical sample was difficult. A sample was collected by chromatography over silica gel, eluting with 80% $CHCl_3$/20% CH_3OH containing a few drops of Et_3N: ^{1}H NMR 8.19 (m, 2 H), 7.67 (m, 2 H), 7.37 (s, 2 H), 3.42 (t, 4 H), 2.90 (s, 6 H), 2.60 (t, 4 H), 2.24 (s, 12 H); mass spectrum, m/z (relative intensity) 408 M^+ (28), 350 (30), 295 (15), 58 (100). Anal. Calcd for $C_{24}H_{32}N_4O_2$: C, 70.59; H, 7.84; N, 13.73. Found:[47] C, 69.01; H, 7.17; N, 13.01.

1,4-Bis[(2-aziridinylethyl)amino]anthracene-9,10-dione (5g). A mixture of **3** (108 mg, 0.44 mmol) and (2-aminoethyl)-aziridine (225 mg, 2.61 mmol) in pyridine (2 mL) was stirred at room temperature for 96 h. The pyridine was removed under a nitrogen stream and the residue quenched with a cold saturated NaCl solution. The aqueous phase was extracted repeatedly with $CHCl_3$ and the solvent partially concentrated on a rotary evaporator. The solution was added to a silica gel column and the column then eluted with 5% CH_3OH/95% $CHCl_3$ to yield the red monosubstituted product and then the blue bis-substitution product **5g** (17 mg, 10%). The sample was recrystallized from $CHCl_3$/petroleum ether to mp 116–118 °C. This bis-aziridine is fairly unstable when exposed to air as an insoluble blue films coats the glassware on evaporation of the chloroform: ^{1}H NMR ($CDCl_3$) 10.90 (t, 2 H), 8.32 (m, 2 H), 7.66 (m, 2 H), 7.29 (s, 2 H), 3.61 (q, 4 H), 2.60 (t, 4 H), 1.80 (m, 4 H), 1.20 (m, 4 H); mass spectrum, m/z (relative intensity) 376 M^+ (100), 320 (16), 264 (12). Anal. ($C_{22}H_{24}N_4O_2$) C, H, N.

1,4-Bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-anthracene-9,10-dione [Ametantrone] (6a = 1). A mixture of **3** (104 mg, 0.43 mmol) and 2-[(2-aminoethyl)amino]ethanol (450 mg, 4.3 mmol) in pyridine (12 mL) was allowed to stir at room temperature for 65 h. Pentane (40 mL) was added, which led to the separation of a blue oily material. TLC analysis (silica gel, 20:4:1 $CHCl_3$/CH_3OH/Et_3N) showed a major blue spot. The oil was dissolved in $CHCl_3$ by addition of a minimum amount of CH_3OH and chromatographed over silica gel. Gradient eluent using 80/20 and 70/30 $CHCl_3$/CH_3OH and then 80/20/1 and 70/30/5 $CHCl_3$/CH_3OH/Et_3N led to various fractions. The last eluates on concentration gave **6a** (52 mg, 30%): mp 155–156 °C [lit.[2b] mp 156–158 °C]; ^{1}H NMR (DMSO-d_6) 10.95 (t, 2 H), 8.25 (m, 2 H), 7.80 (m, 2 H), 7.50 (s, 2 H), 3.50 (m, 8 H), 2.85 (t, 4 H), 2.65 (t, 4 H).

(47) The observed discrepancy between the elemental analysis results may well be due to the hygroscopic nature of this material and its high affinity for atmospheric carbon dioxide. The ^{1}H NMR correlates beautifically with the proposed structure.

1,4-Bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-5,8-dihydroxyanthracene-9,10-dione [Mitoxantrone] (6b = 2). A solution of 2-[(2-aminoethyl)amino]ethanol (231 mg, 2.22 mmol) in pyridine (2 mL) was added to **4** (63 mg, 0.23 mmol) in pyridine (1.5 mL). An immediate purple coloration occurred. The mixture was stirred at room temperature for 50 h and pentane (10 mL) was added. The pentane was removed by decantation and the oil again washed with pentane (5 mL). The crude blue oil was placed under vacuum for 8 h, taken up in 5% MeOH/95% $CHCl_3$, and added to a silica gel column. Gradient elution with 5%, 8%, 10%, 20%, 30%, 40%, and 50% MeOH in $CHCl_3$ led first to 11 (11 mg, 11%; eluting with 30% MeOH/$CHCl_3$): ^{1}H NMR (DMSO-d_6) 14.40 (s, 1 H), 13.55 (br s, 1 H), 11.35 (br s, 1 H), 10.95 (br s, 1 H), 7.0 (m, 2 H), 6.10 (t, 1 H), 2.90 (t, 2 H), 2.65 (t, 2 H), 2.50 (complex m, 4 H). Anal. ($C_{22}H_{28}N_4O_6$) C, H, N. The desired **6b** (30 mg, 30%) was eluted with 50% MeOH/50% $CHCl_3$): ^{1}H NMR ($CDCl_3$ + CD_3OD) 10.4 (s, 2 H), 7.0 (s, 2 H), 6.9 (s, 2 H), 3.75 (m, 4 H), 3.45 (m, 4 H), 3.0 (m, 4 H), 2.90 (m, 4 H).

1,4-Bis[[2-(dimethylamino)ethyl]amino]-5,8-dihydroxyanthracene-9,10-dione (6c). A mixture of **4** (0.10 g, 0.41 mmol) and *N,N*-dimethylethylenediamine (0.18 g, 1.05 mmol) in pyridine (4 mL) was stirred at room temperature for 48 h. Cold brine was added to the reaction mixture and the solid was collected by filtration (0.122 g, 78%). TLC (silica gel, 20% MeOH/80% $CHCl_3$) showed one blue spot. The product was recrystallized from $CHCl_3$/petroleum ether: mp 236–238 °C [lit.[2b] mp 236–238 °C]; ^{1}H NMR ($CDCl_3$) 13.50 (s, 2 H), 10.4 (br s, 2 H), 7.2 (s, 2 H), 7.1 (s, 2 H), 3.50 (q, 4 H), 2.70 (t, 4 H), 2.35 (s, 12 H).

1-[[2-(Dimethylamino)ethyl]amino]-4-fluoroanthracene-9,10-dione (7a). A. Pyridine as Solvent. A mixture of **3** (1.65 g, 6.76 mmol) and 2-(dimethylamino)ethylamine (0.53 g, 6.0 mmol) in pyridine (7 mL) was stirred at room temperature for 21 h. The reaction mixture was poured into a cold saturated sodium chloride solution and the crude solid was collected by filtration and dried. This solid was dissolved in $CHCl_3$ and introduced onto a column of silica gel made up with $CHCl_3$. Initial elution with $CHCl_3$ led to unreacted **3** (0.39 g) while elution with 5% methanol/95% $CHCl_3$ yielded **7a** (0.76 g, 47% yield). An analytical sample was obtained by crystallization from ethanol/water: mp 117–118 °C; ^{1}H NMR ($CDCl_3$) 10.04 (b, 1 H), 8.26 (m, 2 H), 7.77 (m, 2 H), 7.32 (m, 1 H), 7.08 (m, 1 H), 3.45 (q, 2 H), 2.65 (t, 2 H), 2.36 (s, 6 H); mass spectrum, m/z (relative intensity) 312 M^+ (8.6), 254 (6.2), 58 (100); Anal. ($C_{18}H_{17}FN_2O_2$) C, H, N.

B. DMSO as Solvent. Treatment of **3** (330 mg, 1.35 mmol) with 2-(dimethylamino)ethylamine (106 mg, 1.20 mmol) in DMSO (1 mL) for 1 h 45 min followed by a water quench, extraction with $CHCl_3$, and chromatography as in A above led to **7a** (124 mg, 78%).

C. Chloroform as Solvent. Treatment of **3** (100 mg, 0.41 mmol) with 2-(dimethylamino)ethylamine (360 mg, 4.0 mmol) in $CHCl_3$ (2 mL) for 63 h followed by removal of the solvent using a nitrogen stream, addition of water, and filtration led to crude **7a** (111 mg). Chromatography as in A above led to pure **7a** (83 mg, 64%).

1-[(2-Aminoethyl)amino]-4-fluoroanthracene-9,10-dione (7b). A mixture of **3** (140 mg, 0.53 mmol) and 1,2-diaminoethane (40 mg, 0.66 mmol) in pyridine (1 mL) was stirred at room temperature for 23 h. The pyridine was removed under a stream of nitrogen to yield crude **7b** (150 mg). This material was taken up in 5% CH_3OH/95% $CHCl_3$ (not all dissolved) and added to a silica gel column. Gradient elution from 10%, 15%, and 20% CH_3OH in $CHCl_3$ gave a reddish fraction of **7b** (50 mg, 33%): mp 111–113 °C; ^{1}H NMR ($CDCl_3$) 10.05 (m, 1 H), 8.2 (m, 2 H), 7.70 (m, 2 H), 7.3 (t, 1 H), 7.0 (m, 1 H), 3.40 (m, 2 H), 3.05 (br s, 2 H), 1.20 (br s, 2 H). Anal. ($C_{16}H_{13}FN_2O_2$) C, H, N.

1-[Methyl[2-(dimethylamino)ethyl]amino]-4-fluoroanthracene-9,10-dione (7c). A mixture of **3** (100 mg, 0.41 mmol) and *N,N,N'*-trimethylethylenediamine (47 mg, 0.46 mmol) in pyridine (1 mL) was stirred at room temperature for 17 h. The pyridine was evaporated under a slow stream of nitrogen and the residue quenched with a cold saturated NaCl solution. The product was extracted with $CHCl_3$ (3 × 20 mL) and the solvent removed under vacuum. Purification by silica gel chromatography using gradient elution of 5%, 10%, 50% CH_3OH in $CHCl_3$ and then CH_3OH led to red fractions; which on concentration gave **7c** (90 mg, 67%) as a gummy solid that resisted attempts at

crystallization: ^{1}H NMR ($CDCl_3$) 8.18 (m, 2 H), 7.70 (m, 2 H), 7.46 (m, 1 H), 7.28 (m, 1 H), 3.49 (t, 2 H), 2.94 (s, 3 H), 2.58 (t, 2 H), 2.21 (s, 6 H); mass spectrum, m/z (relative intensity) 326 M^+ (18), 268 (9), 184 (20), 58 (100). Anal. ($C_{19}H_{19}FN_2O_2$) C, H, N.

1-[(2-Hydroxyethyl)amino]-4-fluoroanthracene-9,10-dione (7d). A solution of **3** (101 mg, 0.41 mmol) and 2-aminoethanol (250 mg, 4.1 mmol) in $CHCl_3$ (2 mL) was stirred at room temperature for 17 h. The mixture was concentrated by using a slow nitrogen stream, cold water was added, and the solid was collected by filtration and dried (100 mg). The sample was dissolved in $CHCl_3$ and added to a silica gel column. A small amount of unreacted **3** eluted. Elution with 2% CH_3OH/98% $CHCl_3$ gave a purple fraction, which on concentration gave **7d** (58 mg, 50%): mp 142–143 °C; ^{1}H NMR ($CDCl_3$) 10.10 (m, 1 H), 8.20 (m, 2 H), 7.70 (m, 2 H), 7.30 (1 H), 7.10 (1 H), 4.00 (m, 2 H), 3.55 (m, 2 H). Anal. ($C_{16}H_{12}FNO_2$) C, H, N.

1-Aziridinyl-4-fluoroanthracene-9,10-dione (7e). A solution of **3** (108 mg, 0.44 mmol) and aziridine (236 mg, 5.5 mmol) in pyridine (2 mL) was allowed to stir for 3 h. The bright yellow solid that precipitated was filtered and washed thoroughly with pentane to yield **7e** (50 mg, 19%): mp 208–210 °C dec; ^{1}H NMR ($CDCl_3$) 8.25 (m, 2 H), 7.78 (m, 2 H), 7.40 (m, 2 H), 1.40 (s, 4 H); mass spectrum, m/z (relative intensity) 267 M^+ (30), 238 (100), 183 (26). Anal. ($C_{16}H_{10}FNO_2$) C, H, N.

1-[(2-Aziridinylethyl)amino]-4-fluoroanthracene-9,10-dione (7f). A mixture of **3** (109 mg, 0.45 mmol) and (2-aminoethyl)aziridine (357 mg, 4.1 mmol) in $CHCl_3$ (2 mL) was stirred at room temprature for 28 h. The mixture was concentrated under a nitrogen stream, quenched into ice water, and filtered to yield a purple solid (62 mg). This crude material was dissolved in $CHCl_3$ and placed on a silica gel chromatographic column. Elution with 1% CH_3OH/99% $CHCl_3$ led to several reddish fractions, which on concentration led to **7f** (35 mg, 24%): mp 143–145 °C; ^{1}H NMR ($CDCl_3$) 10.10 (b, 1 H), 8.20 (m, 2 H), 7.7 (m, 2 H), 7.35 (m, 1 H), 7.15 (m, 1 H), 3.5 (m, 2 H), 2.54 (t, 2 H), 1.81 (slightly split s, 2 H), 1.22 (slightly split s, 2 H); mass spectrum, m/z (relative intensity) 310 M^+ (61), 254 (100), 56 (32). Anal. ($C_{18}H_{15}FN_2O_2$) C, H, N.

1-[[2-(Dimethylamino)ethyl]amino]-4-fluoro-5,8-dihydroxyanthracene-9,10-dione (8). Compound **4** (54 mg, 0.19 mmol) and *N,N*-dimethylethylenediamine (22 mg, 0.25 mmol) were placed in pyridine (3 mL) and the mixture was stirred at room temperature for 28 h. The mixture was quenched into a cold saturated solution of NaCl and filtered to yield a crude purple solid (37 mg). This was purified by column chromatography (silica gel, gradient elution from $CHCl_3$ to 5% CH_3OH in $CHCl_3$) to yield **8** (28 mg, 42%). Recrystallization was effected from $CHCl_3$/high boiling petroleum ether: mp 153–156 °C; ^{1}H NMR ($CDCl_3$) 13.05 (s, 2 H), 9.81 (br s, 1 H), 7.37 (m, 1 H), 7.22 (s, 1 H), 7.20 (s, 1 H), 7.13 (dd, 1 H), 3.50 (m, 2 H), 2.72 (t, 2 H), 2.34 (s, 6 H); mass spectrum, m/z (relative intensity) 344 M^+ (3), 286 (2), 202 (5), 58 (100). Anal. $C_{18}H_{17}F_2N_2O_4$) C, H, N.

1-[(2-Aminoethyl)amino]-4-[[2-(dimethylamino)ethyl]amino]anthracene-9,10-dione (9a). A solution of **7a** (512 mg, 1.63 mmol) and ethylenediamine (660 mg, 11 mmol) in pyridine (2 mL) was stirred at room temperature for 20 h. Low boiling petroleum ether (7 mL) was added and the precipitated blue solid was filtered and dried under vacuum (500 mg, 87%). Crystallization attempts from $CHCl_3$ and low boiling petroleum ether yielded an amorphous blue solid: mp 123–125 °C; ^{1}H NMR ($CDCl_3$) 10.90 (m, 2 H), 10.80 (m, 1 H), 8.35 (m, 2 H), 7.70 (m, 2 H), 7.20 (m, 2 H), 3.50 (m, 4 H), 3.10 (t, 2 H), 2.70 (t, 2 H), 2.30 (s, 6 H); mass spectrum, m/z 352 M^+. Anal. Calcd for $C_{20}H_{24}N_4O_2$: C, 68.17; H, 6.86; N, 15.90. Found: C, 68.56; H, 6.08; N, 14.10.[47]

1-[[2-(Dimethylamino)ethyl]amino]-4-[[2-[(2-hydroxyethyl)amino]ethyl]anthracene-9,10-dione (9b). A solution of 2-[(2-aminoethyl)amino]ethanol (1.6 g, 15.4 mmol) in DMSO (8 mL) was added to **7a** (454 mg, 1.8 mmol) in DMSO (1 mL). The mixture was stirred at room temperature for 27 h and poured into a cold saturated NaCl solution. The product was extracted with $CHCl_3$, the extracts were dried over sodium sulfate, and the solvent was removed under reduced pressure. Low boiling petroleum ether was added to the residual material and the blue solid **9b** was collected by filtration (650 mg, 94%). The TLC of this crude material showed essentially one deep blue spot ($CHCl_3$/CH_3OH 4:1 with a trace of Et_3N). The material was dissolved in a 4:1 $CHCl_3$/CH_3OH solution and placed on a silica gel column. Pure **9b** was eluted with this solvent mixture containing 1% Et_3N. A sample was also crystallized from ethanol/hexane: mp 115–116 °C; ^{1}H NMR 10.93 (b, 1 H), 10.73 (b, 1 H), 8.32 (m, 2 H), 7.68 (m, 2 H), 7.25 (s, 2 H), 3.73 (m, 2 H), 3.55 (m, 4 H), 3.05 (m, 2 H), 2.90 (m, 2 H), 2.69 (m, 2 H), 2.40 (s, 6 H); mass spectrum, m/z (relative intensity) 396 M^+ (3), 351 (9), 277 (2), 65 (6), 74 (36), 58 (100). Anal. $C_{22}H_{28}N_4O_3$), C, H, N.

1-[(4-Aminobutyl)amino]-4-[[2-(dimethylamino)ethyl]amino]anthracene-9,10-dione (9c). A pyridine solution (1 mL) containing 1,4-diaminobutane (93 mg, 1.05 mmol) was added to **7a** (110 mg, 0.35 mmol) and the mixture was stirred for 24 h. The pyridine was removed under a nitrogen stream and the residue placed under vacuum for a day. The product was purified on a chromatotron [silica gel plate with elution by $CHCl_3$/Et_3N, 50/1]. Concentration of the solvent afforded a blue solid (0.12 g, 90%): mp 122–125 °C [lit.[3] mp 88–90 °C]; ^{1}H NMR ($CDCl_3$) 10.80 (br, 2 H), 8.35 (m, 2 H), 7.69 (m, 2 H), 7.23 (s, 2 H), 3.46 (m, 4 H), 2.78 (t, 2 H), 2.70 (t, 2 H), 2.35 (s, 6 H), 1.82 (m, 2 H), 1.68 (m, 2 H).

1-[[2-(Dimethylamino)ethyl]amino]-4-[methyl[2-(dimethylamino)ethyl]amino]anthracene-9,10-dione (9d). A pyridine solution (1 mL) of **7a** (107 mg, 0.34 mmol) and *N,N,N'*-trimethylethylenediamine (34 mg, 1.3 mmol) was stirred at room temperature for 40 h. The pyridine was allowed to evaporate in the hood and the residue treated with a cold brine solution. The mixture was extracted with methylene chloride, and the extracts were dried over sodium sulfate and concentrated to yield crude **9d** (128 mg). The oil was dissolved in $CHCl_3$ and placed on a silica gel column. Gradient elution from 5% CH_3OH to 50% CH_3OH in $CHCl_3$ and then 49/49/1 CH_3OH/$CHCl_3$/NH_4OH led to a blue solid (40 mg, 45%); mp 87–89 °C; ^{1}H NMR ($CDCl_3$) 10.40 (t, 1 H), 8.30 (m, 2 H), 7.72 (m, 2 H), 7.50 (d, 1 H), 7.10 (d, 1 H), 3.45 (m, 4 H), 2.90 (s, 3 H), 2.70 (t, 2 H), 2.60 (t, 2 H), 2.40 (s, 6 H), 2.25 (s, 6 H). Anal. ($C_{23}H_{30}N_4O_2$) C, H, N.

1-[(4-Aminobutyl)amino]-4-[[2-(dimethylamino)ethyl]amino]-5,8-dihydroxyanthracene-9,10-dione (10a). A mixture of **8** (0.05 g, 0.15 mmol) and 1,4-diaminobutane (0.3 mL, 3 mmol) in pyridine (0.5 mL) was stirred at room temperature for 18 h. The addition of petroleum ether precipitated a blue solid (37 mg, 59%): mp 156–157 °C; ^{1}H NMR ($CDCl_3$) 13.51 (br s, 2 H), 10.4 (b, 2 H), 7.10 (m, 4 H), 3.44 (m, 4 H), 2.77 (m, 4 H), 2.5 (s, 6 H), 1.72 (m, 4 H); mass spectrum, m/z (relative intensity) 412 M^+ (24), 367 (12), 283 (12), 70 (36), 58 (100). Anal. ($C_{22}H_{28}N_4O_4$) C, H, N.

1-[[2-(Dimethylamino)ethyl]amino]-4-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-5,8-dihydroxyanthracene-9,10-dione (10b). Fluoro analogue **8** (72 mg, 0.21 mmol) and 2-[(2-aminoethyl)amino]ethanol (0.21 mL, 218 mg, 2.1 mmol) in pyridine (3 mL) were stirred at room temperature for 16 h. The mixture was poured into saturated brine and extracted with chloroform, the chloroform extract was back extracted with brine, and the chloroform solution was dried over Na_2SO_4. Chromatography (silica gel, gradient elution from 100% $CHCl_3$ to 1:40:10 triethylamine/$CHCl_3$/MeOH) of the reaction product gave **10b** (44 mg, 48%). The compound was recrystallized from $CHCl_3$/ligroin; mp 188–190 °C; ^{1}H NMR ($CDCl_3$) 13.54 (b, 2 H), 10.38 (br t, 1 H), 10.25 (br t, 1 H), 7.15 (s, 2 H), 7.00 (s, 2 H), 3.80 (m, 2 H), 3.45 (m, 4 H), 3.0 (t, 2 H), 2.85 (t, 2 H), 2.65 (t, 2 H), 2.35 (s, 6 H). Anal. ($C_{22}H_{28}N_4O_5 \cdot H_2O$) C, H, N.

Biological Studies. In Vitro Cytotoxicity Evaluations. L1210 murine leukemia cells are routinely maintained as suspension cultures in McCoy's 5A medium supplemented with 10% horse serum, glutamine, penicillin, and streptomycin and grown in a humidified environment of 10% carbon dioxide and 90% air at 37 °C. For assessment of the in vitro toxicity, each compound was dissolved in dimethyl sulfoxide and added to 1 mL of L1210 cells (5×10^4 cells/mL) to attain final concentrations of 0.01, 0.1, and 10 μg of drug/mL of culture. After 72 h of continuous exposure to the drug, the cell concentration was determined with a Coulter counter. Growth inhibition was calculated for each drug concentration by using the following formula:

$$\% \text{ growth inhibition} = \left[1 - \left(\frac{\text{cell number treated}}{\text{cell number DMSO alone}}\right)\right]100$$

The growth inhibition data were then used to calculate the ID_{50} values (the drug concentration required to inhibit cell growth by 50% of control).

LOVO[48] and LOVO/DOXO[49] were cultured at 37 °C by using Ham's F12 medium supplemented with 20% fetal calf serum and maintained at 37 °C in an atmosphere of 5% carbon dioxide. LOVO and LOVO/DOXO were plated at concentrations of 2.5×10^3 cells/dish. After 144 h of continuous drug exposure, growth inhibition was evaluated (ID_{50}) via an MTT assay.

In Vivo Efficacy Studies. L1210, P388, and P388/DOXO murine leukemia cells are maintained in vivo by weekly intraperitoneal (ip) injections of 10^6 cells, respectively, in BDF_1, DBA_2, and CDF_1 mice. For test purposes, mice were inoculated ip with 10^6 tumor cells and treatment was initiated 24 h later. The desired dose of drug was administered on days 1, days 1 and 5, or days 1, 5, and 9 as reported in the tables. Mice were observed daily for signs of toxicity and survival. The day of death was recorded for all animals that died or were sacrificed during the 60-day study group. The mean survival time (MST) for each treatment group was calculated and the percent T/C was determined by using the following formula:

$$\%T/C = [(\text{MST treated})/(\text{MST control})]100$$

Registry No. 1, 64862-96-0; 2, 65271-80-9; 3, 28736-42-7; 4, 131401-54-2; 5a, 2944-12-9; 5b, 134529-39-8; 5e, 4471-41-4; 5d, 19853-95-3; 5e, 69895-68-7; 5f, 134529-40-1; 5g, 134529-43-4; 6c, 70476-63-0; 7a, 134529-36-5; 6b, 134529-44-5; 7c, 134529-45-6; 7d, 134529-46-7; 7e, 134529-47-8; 7f, 134529-48-9; 8, 134529-37-6; 9a, 129732-44-1; 9b, 121498-41-7; 9c, 102650-22-6; 9d, 134529-41-2; 10a, 134566-65-7; 10b, 134529-42-3; 11, 134529-38-7; $PhNH_2$, 62-53-3; $HOCH_2CH_2NH_2$, 141-43-5; $NH_2CH_2CH_2NH_2$, 107-15-3; $NH_2CH_2CH_2N(CH_3)_2$, 108-00-9; $CH_3NHCH_2CH_2N(CH_3)_2$, 142-25-6; $NH_2CH_2CH_2NHCH_2CH_2OH$, 111-41-1; $NH_2(CH_2)_4NH_2$, 110-60-1; cyclopropylamine, 765-30-0; 1-(2-aminoethyl)aziridine, 4025-37-0; aziridine, 151-56-4.

Supplementary Material Available: In vivo antitumor activity of 10a evaluated against P388 murine leukemia (Table VI), P388/DOXO resistant murine leukemia (Table VII), and on human mammary carcinoma (MX-1) transplanted in nude mice (Table VIII) (3 pages). Ordering information is given on any current masthead page.

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Synthesis and Antiviral Activity of 3-Substituted Derivatives of 3,9-Dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purines, Tricyclic Analogues of Acyclovir and Ganciclovir

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9-[(2-Hydroxyethoxy)methyl]guanine (acyclovir, 1a) and 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, ganciclovir, 1b) were transformed to their respective tricyclic derivatives, 3-substituted 3,9-dihydro-9-oxo-5*H*-imidazo[1,2-*a*]purines 2b, 3a, and 3b. The 6-methyl-substituted compound 2b was obtained following reaction of 1b with bromoacetone. A two-step approach via 1-(2,2-diethoxyethyl) intermediates 4a,b was the most effective for the preparation of the derivatives unsubstituted in the appended ring (3a,b). The novel acyclonucleosides, in particular ganciclovir derivative 2b, proved markedly active against herpes simplex virus type 1 and 2, varicella-zoster virus, and cytomegalovirus.

During our previous work on the antiviral activity of novel N-substituted derivatives of acyclovir, 9-[(2-hydroxyethoxy)methyl]guanine (1a), we found that the tricyclic 3,9-dihydro-3-[(2-hydroxyethoxy)methyl]-6-methyl-9-oxo-5*H*-imidazo[1,2-*a*]purine (2a)[1] exhibited marked and selective activity against herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2). We have now extended these studies to additional tricyclic analogues bearing either a 3-[(1,3-dihydroxy-2-propoxy)methyl] side chain (2b) or a 6-unsubstituted appended imidazole ring (3a), or both (3b).

The discussed tricyclic ring system is frequently referred to in the literature as 1,*N*-2-ethenoguanine to indicate its relation to the parent, naturally occuring heterocycle.

Chemistry

The tricyclic analogue of ganciclovir, 3,9-dihydro-3-[(1,3-dihydroxy-2-propoxy)methyl]-6-methyl-9-oxo-5*H*-imidazo[1,2-*a*]purine (2b), was obtained upon reaction of the 1-sodium derivative of 1b in dimethylformamide with bromoacetone (yield 84%) according to a previously reported method for the preparation of the analogously modified guanosine (2c)[2-4] and acyclovir (2a) (Scheme I).[1]

Literature data on the formation of 1,*N*-2-etheno derivatives of guanine have been limited to the report of Sattsangi et al.[5] The authors obtained 1,*N*-2-ethenoguanosine (3c) in 7% yield after reacting guanosine with aqueous chloroacetaldehyde under physiological conditions. Three approaches toward a more efficient synthesis of 3c have been recently developed.[6] They are based upon the reaction of guanosine with (i) aqueous chloroacetaldehyde at pH 10, (ii) anhydrous haloacetaldehydes, or (iii) bromoacetaldehyde diethyl acetal.

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