6,9-Bis[(aminoalkyl)amino]benzo[g]isoquinoline-5,10-diones. A Novel Class of Chromophore-Modified Antitumor Anthracene-9,10-diones: Synthesis and Antitumor Evaluations

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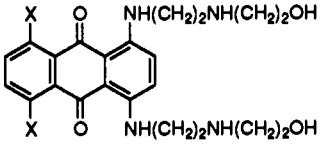
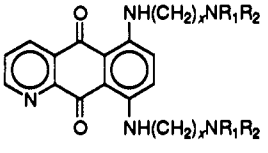
Synthetic procedures have been developed which lead to the 2-aza congeners **3** and several related *N*-oxides **4**. The analogues **3** exhibited a wide range of *in vitro* cytotoxicity against L1210 leukemia, the human colon adenocarcinoma cell line LoVo, and the doxorubicin resistant LoVo/DX cell line. Selected analogues of **3** showed significant P388 antileukemic activity in mice with **3c** exhibiting high activity. This activity was also retained in the related *N*-oxide **4a**. These heterocyclic bioisosteric models are representative of the first anthracene-9,10-diones which display antileukemic activity comparable to mitoxantrone.

Introduction

Ametantrone (**1a**) and mitoxantrone (**1b**) are among the most representative examples of the antitumor 1,4-bis(aminoalkylamino)anthracene-9,10-diones.[1,2] Mitoxantrone, in particular, is currently gaining an important place in the clinical management of leukemias and lymphomas as well as in combination therapy of advanced breast and ovarian cancers.[2c] Although the toxic side effects associated with mitoxantrone chemotherapy appear to be less pronounced than in the case of doxorubicin, myelotoxicity and cardiotoxicity still appear to be of clinical concern, especially in patients previously treated with doxorubicin.[2c] Moreover, development of resistance limits the therapeutic potential of this drug. Therefore, the need still exists for anthracene-9,10-dione congeners endowed with improved therapeutic efficacy and less toxic side effects, as well as effectiveness against multiple drug-resistant (MDR) cell lines.[3]



1a: X = H
b: X = OH



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As in the anthracyclines, the cytotoxic effects of **1a** and **1b** are probably multimodal. Many studies suggest that intercalation into DNA is a major cellular event, and this interaction may serve as an "anchor" for the drugs at specific base pair sites which is then followed by the critical cell killing events.[4] Biophysical and biochemical studies have led to reasonably clear structural representations of the DNA–anthracene-9,10-dione intercalation complexes.[5,6]

Nucleic acid condensations[7] and free radical intermediates[8-10] have also been considered as contributors to the cell killing effects of **1a** and **1b**.

Recently it has been proposed that the antitumor activities of the DNA intercalators such as **1a** and **1b** are due to the disruption of DNA protein interactions, in particular the interference of topoisomerase II.[11] One can consider these drugs to consist of a DNA intercalation domain (anthracene-9,10-dione region) and a protein binding domain (side arms).[12]

In the search for analogues with optimal therapeutic efficacy, the primary structural changes introduced into the anthracene-9,10-dione chemotypes have been (a) variation of the alkylamino side chain and (b) repositioning of the hydroxy substituents and/or the alkylamino side chains of the anthracenedione chromophore.[1b,13] This latter effect has led to compounds endowed with very potent cytotoxicity. However, the 5,8-dihydroxy substitution pattern found in mitoxantrone (**1b**) also seems to be involved in the delayed lethality observed in animals treated with this drug but not with ametantrone (**1a**).[14]

The study of congeners with heteroatoms in the anthracene-9,10-dione chromophore appears to be a relatively unexplored area.[15] Heterocyclic analogues related to **1a** and **1b** (heteroannulated naphthoquinones) (a) could potentially retain the same spatial and planar characteristics for host molecular recognition such as DNA intercalation and (b) could be endowed with sites of hydrogen bonding or basic sites, possibly increasing the affinity of the drug for DNA and/or affecting the interaction with topoisomerase II. In particular the presence of nitrogen atom(s) in the ring might lead to molecules which intercalatively bind with DNA with greater affinity than the carbocyclic analogues with a resultant potentially stronger interaction with topoisomerase II.[16]

Although a prior study revealed that the 1-aza bioisoteric models **2** exhibited modest *in vivo* activities in L1210 screening,[17a] our group has systematically investigated the introduction of nitrogen atoms into different positions of the anthracene-9,10-dione nucleus as a tool for the discovery of second-generation anthracenedione analogues. This effort resulted in the preparation of the aza and diaza analogues[17a-i] depicted in Chart 1.

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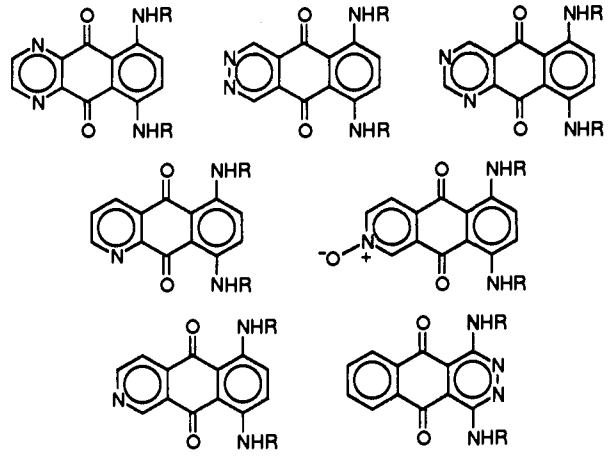
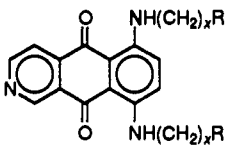
Chart 1

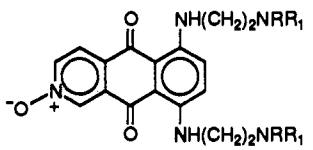


In this paper we report the synthesis and biological evaluations of a set of novel 6,9-bis(aminoalkylamino)-benzo[*g*]isoquinoline-5,10-diones(2-azaanthracenediones) **3a–d,f–j,l–t**[17e] and two related *N*-oxides **4a,b**.[17f] This work represents a significant part of our research which led to the identification of compound **3c**, a second-generation, well-tolerated anthracenedione analogue endowed with anticancer activity superior (leukemias) or at least comparable (solid tumors) to that of mitoxantrone.[17g,h,i]



3

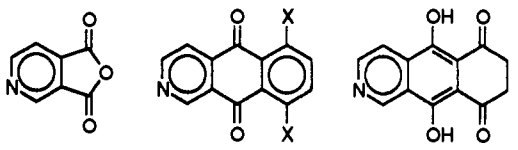
a: $x = 2$, R = $N(CH_3)_2$
b: $x = 2$, R = N-c-C_2H_4
c: $x = 2$, R = NH_2
d: $x = 2$, R = $NHCOCH_3$
e: $x = 2$, R = $NHCO_2{}^tBu$
f: $x = 2$, R = $NHCH_2CH_2OH$
g: $x = 3$, R = NH_2
h: $x = 4$, R = NH_2
i: side chains = $NHCH_2C(CH_3)_2NH_2$
j: $x = 2$, R = $NHCH_2CH_2OCH_3$
k: $x = 2$, R = $N(CH_3)CO_2{}^tBu$
l: $x = 2$, R = $NHCH_3$
m: $x = 2$, R = $NHCH_2CH_3$
n: $x = 2$, R = $NHCH_2CH_2CH_3$
o: $x = 2$, R = $NHCH(CH_3)_2$
p: $x = 2$, R = $N(CH_2CH_3)_2$
q: $x = 2$, R = $N[CH(CH_3)_2]_2$
r: $x = 2$, R = N(pyrrolidine)
q: $x = 2$, R = N(morpholine)
t: $x = 3$, R = $N(CH_3)_2$
u: $x = 2$, R = OCH_2CH_2OH



4a: $R = R_1 = H$
b: $R = R_1 = CH_3$

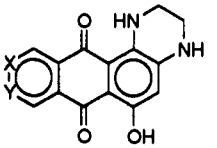
Synthesis

Two synthetic pathways were explored for the preparation of the analogues related to **3**. In the first method, the Friedel–Crafts bis-acylation of 1,4-dimethoxybenzene with pyridine-3,4-dicarboxylic anhydride (**5**) and molten aluminum chloride/sodium chloride led to the 2-aza compound **6a** (20%).[18] This compound was reduced to the leuco form **7** by treatment with sodium dithionite. The structure of the reduced product as **7** is based on ^1H NMR and ^{13}C NMR analyses.[19] The ^{13}C NMR resonances for the carbonyls and the adjacent CH_2 groups are 201.5, 200.7, and 35.8, 36.1 ppm, respectively.



5 **6a**: X = OH **b**: X = F **7**

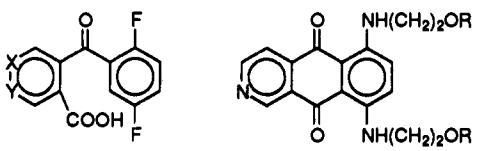
The *in situ* reduction of **6a** to **7** followed by addition of the appropriate amine and air oxidation during the workup led to the 6,9-bis[(aminoalkyl)amino]benzo[*g*]isoquinoline-5,10-diones **3a** (38%), **3b** (5%), **3c** which resisted crystallization (26%, as the hydrochloride salt), **3d** (48%, from acetylation of **3c**), and **3e** (48%). The byproducts isolated from the reaction mixture which led to **3c** were the undesired cyclic regioisomers **8a** and **8b** (two singlets at δ 6.03 and 6.11 for the aromatic proton of each adjacent to the OH group). The use of *N*-(*tert*-butoxycarbonyl)-ethylenediamine[20] in the condensation with **7** avoided the formation of these products. Deprotection of **3e** with dry hydrogen chloride gas led to **3c** as the hydrochloride salt. This salt was hygroscopic and unstable and decomposed on standing to unidentified products.



8a: X = N, Y = CH
b: X = CH, Y = N

Attempts to prepare the ametantrone analogue **3f** by treatment of leuco **7** with 2-((2-aminoethyl)amino)ethanol were frustrated by isolation difficulties.

In the second methodology, we turned our efforts toward the synthesis of **6b**.[13] A mixture of keto acids **9a** and **9b** was obtained in the reaction of pyridine-3,4-dicarboxylic anhydride (**5**) with 1,4-difluorobenzene in the presence of aluminum chloride.[21] An alternative procedure to the regioisomeric mixture of keto acids **9a** and **9b** was by reaction of 2-lithio-1,4-difluorobenzene[22] with anhydride **5**. Treatment of the mixture of keto acids from each reaction with ethereal diazomethane led to the corresponding methyl esters. These crude esters on ^1H NMR analysis exhibited singlets at δ 3.82 and 3.78 for the methyl groups of the esters in a 4:1 ratio, respectively. It is of interest to note that the ratio of **9a**:**9b** was about 4:1 from either method of synthesis. The major regioisomer found in the directed metalation reaction was formed by attack at the more electrophilic carbonyl center.[23]



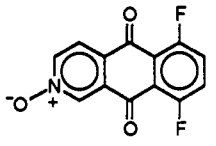
9a: X = CH, Y = N **b**: X = N, Y = CH **10a**: R = H **b**: R = Ms

Initial attempts to cyclize this keto acid mixture to **6b** with concentrated sulfuric acid or polyphoshporic acid were unsuccessful. However, the use of fuming sulfuric acid (30%)[21] at 140 °C for 6 h led to excellent yields of **6b** (70–80%).

Treatment of **6b** with the appropriate diamines in pyridine (or chloroform in a few cases) at room temperature led to the desired analogues **3a,c,f–i,m–t**. The HPLC

purity of **3c** never exceeded 96.5% (as area %) due to the presence of unknown impurities with higher retention times. For antitumor studies, **3c** as well as **3f–h,r** were converted to the easily crystallizable and water-soluble dimaleate salts. It should also be noted that even in the case of its dimaleate salt the HPLC purity of **3c** never exceeded 96.1% (as area %) due to the presence of the above-mentioned impurities. Many attempts to purify **3c** or its dimaleate salt by column chromatography or crystallization have thus far been unsuccessful. Treatment of **6b** with the appropriate BOC-protected diamines led to **3e** and **3k**, respectively. Treatment of **3k** with ethanolic hydrogen chloride led to the hydrochloride salt **3l**. Analogue **3u** was prepared by treatment of **6b** with the appropriate monoamine. In comparable cases, the yields of **3** from the difluoro analogue **6b** were much higher than those obtained from leuco **7**.

An alternative preparation of **3f** involved buildup of the side arm of diol **10a** which was readily prepared by reaction of 2-aminoethanol with **6b** in pyridine in 94% yield. Treatment of **10a** with methanesulfonyl chloride gave **10b**. As a model to establish that the displacements of the mesylate groups of **10b** would occur, dimethylamine was bubbled through a pyridine solution of **10b** to prepare **3a** (76%). Congener **3j** was prepared in a 66% yield by treatment of **10b** with 20 equiv of 2-methoxyethylamine at room temperature for 48 h. When 2-aminoethanol was used under the same conditions, a very polar blue spot was detectable by TLC (silica gel), indicating the presence of **3f**. However, during the workup, **3f** could not be separated from the excess ethanolamine. The protected 2-(trimethylsiloxy)ethylamine was utilized in the displacement reaction with **10b**. During purification by column chromatography over silica gel, the O–Si bond was cleaved to yield **3f**. Because of its hygroscopic nature, **3f** was converted into the maleic acid salt. The overall conversion from the mesylate to the dimaleate salt was accomplished in a 40% yield. The *N*-oxide analogues **4a** and **4b** were prepared by treatment of *N*-oxide **11** with ethylenediamine or *N,N*-dimethylethylenediamine, respectively. Compound **4a** was converted into its dimaleate salt. The preparation of **11** was accomplished by treatment of **6b** with *m*-chloroperoxybenzoic acid in dichloromethane.



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Biological Evaluations

The evaluations of the biological activity for the compounds were performed *in vitro* and *in vivo* following the protocols developed by the National Cancer Institute. The *in vitro* activities against L1210 murine leukemia for the 2-aza chemotypes along with comparative data for ametantrone (**1a**) and mitoxantrone (**1b**) are listed in Table 1.

The cytotoxic activity of a number of the analogues **3** and of the two related *N*-oxides **4a** and **4b** were evaluated against the human colon adenocarcinoma cell line LoVo and its subline resistant to doxorubicin LoVo/Dx. The results are tabulated in Table 2 which also presents comparative cytotoxic activity for doxorubicin, ametantrone (**1a**), and mitoxantrone (**1b**).

Table 1. *In Vitro* Activities of Analogues 3 against L1210 Leukemia

compd	x	R	IC_{50}, μg/mL
		Primary Amino Side Chains	
3c	2	NH_2[a]	0.01
3g	3	NH_2[b]	0.10
3h	4	NH_2	1.5
		Nonbasic Amide Side Chain	
3d	2	$NHCOCH_3$	>10
		Secondary Amino Side Chains	
3f	2	$NH(CH_2)_2OH$[b]	0.06
3j	2	$NH(CH_2)_2OCH_3$	0.44
3m	2	$NHCH_2CH_3$	0.01
3n	2	$NH(CH_2)_2CH_3$	0.04
3o	2	$NHCH(CH_3)_2$	0.03
		Tertiary Amino Side Chains	
3a	2	$N(CH_3)_2$	0.006
3b	2	N-c-C_2H_4	0.002
3p	2	$N(CH_2CH_3)_2$	0.06
3q	2	$N[CH(CH_3)_2]_2$	1.8
3r	2		0.09
3s	2		1.1
3t	3	$N(CH_3)_2$	0.30
ametantrone (1a)			0.03
mitoxantrone (1b)			0.009

[a] Hydrochloride salt. [b] Maleate salt.

Table 2. *In Vitro* Cytotoxic Activity of Analogues 3 and 4 in Comparison with Ametantrone (1a), Mitoxantrone (1b), and Doxorubicin (Dx) on Human Colon Adenocarcinoma Cell Lines Sensitive (LoVo) and Resistant to Doxorubicin (LoVo/Dx)

			IC_{50}, μg/mL (SD)[a]		
compd	x	R	LoVo	LoVo/Dx	RI[b]
		Primary Amino Side Chains			
3c[c]	2	NH_2	0.24(0.12)	7.2(2.5)	30
3g[c]	3	NH_2	2.1(0.33)	54.5(5.3)	26
3h	4	NH_2	2.27(1.13)	0.83(0.11)	0.4
3j		$[CH_2C(CH_3)_2NH_2]$	0.16(0.02)	15.2(2.8)	95
		Secondary Amino Side Chains			
3f[c]	2	$NH(CH_2)_2OH$	1.3(0.3)	99.5(21.4)	76
3l[d]	2	$NHCH_3$	0.08(0.01)	2.9(0.4)	36
3n	2	$NH(CH_2)_2CH_3$	0.2(0.05)	2.8(0.2)	14
3o	2	$NHCH(CH_3)_3$	0.3(0.2)	3.4(0.5)	11
		Tertiary Amino Side Chains			
3a	2	$N(CH_3)_2$	0.076(0.02)	0.13(0.05)	1.7
3p	2	$N(CH_2CH_3)_2$	0.29(0.05)	0.69(0.1)	2.4
3q	2	$N[CH(CH_3)_2]_2$	2.5(0.8)	2.8(0.4)	1.1
3r[c]	2		0.5(0.2)	0.7(0.2)	1.4
3s	2		5.32(1.55)	2.3(0.67)	0.4
3t	3	$N(CH_3)_2$	0.15(0.07)	0.36(0.04)	2.4
		N-Oxides			
4a[c]			7.3(7.8)	62.6(79.5)	9–10
4b			0.44(0.55)	2.6(2.6)	5–6
		Nonbasic Side Chain			
3u	2	$O(CH_2)_2OH$	8.7(3.4)	266.6	31
		Reference Compounds			
1a			0.4(0.2)	34.7(14.1)	87
1b			0.009(0.005)	0.25(0.09)	28
Dx			0.03(0.01)	4.0(1.5)	133

[a] Inhibiting concentration of 50% cellular growth (standard deviation). [b] Resistance index: IC_{50} of resistant cell line/IC_{50} of sensitive cell line. Compounds **3i**, **3t**, and **4a** were dissolved in DMSO, **3a**, **3h**, **3n**, **3o**, **3p**, **3q**, **3s**, and **3u** in citric acid (1%). All the other compounds, **1a**, **1b**, and **Dx** were dissolved in distilled water. All compounds and standards after dissolution were further diluted in complete culture medium. [c] Maleate salt. [d] Hydrochloride salt.

The *in vivo* antitumor activities of selected analogues of **3** and of the two related *N*-oxides **4a** and **4b** were assessed

Table 3. Antitumor Activity of Selected Analogues of 3, 4, Ametantrone (1a), Mitoxantrone (1b), and Doxorubicin (Dx) against P388 Murine Leukemia (iv/iv + 1, 4, 7)[a]

compd	x	R	dose,[b] mg/kg/day	% T/C[c] (range)
		Primary Amino Side Chains		
3c[d]	2	NH_2	27	230 (181–275)
3g[d]	3	NH_2	40	155
3h	4	NH_2	35	100
3i		$[CH_2C(CH_3)_2NH_2]$	40	122
		Secondary Amino Side Chains		
3f[c]	2	$NH(CH_2)_2OH$	40	118
3l	2	$NHCH_3$	8	189
		Tertiary Amino Side Chains		
3a	2	$N(CH_3)_2$	40	183, 156
3p	2	$N(CH_2CH_3)_2$	60	122
3r[d]	2		60	100
3s	2		60	122
4a[d]			60	200, 225
4b			40	122
ametantrone (1a)			58	127
mitoxantrone (1b)			3	196 (167–250)
Dx			7.5	181, 211

[a] 10^6 cells/mouse iv in CD2F1. Treatment iv on days 1, 4, 7 after tumor transplantation (day 0). [b] Maximum tolerated dose (LD_{10}) or maximum administered dose. [c] Median survival time of treated mice/median survival time of controls × 100. [d] Maleate salt.

Table 4. Antitumor Activity of 3c in Comparison with Mitoxantrone (1b) and Doxorubicin (Dx) against P388 Murine Leukemia (iv/iv + 1, 4, 7)[a]

compd	dose, mg/kg/day	% T/C[b] (range)	tox[c]
3c[d]	8	162	0/8
	12	194, 145	0/16
	18	206 (188–225)	1/40
	22	250	1/8
	27	230 (181–275)	7/55
	40	128 (36–278)	25/37
	60	36, 18	15/16
mitoxantrone (1b)	2	172 (154–212)	0/82
	3	196 (167–250)	12/132
	4	138 (89–162)	43/48
Dx	6	175, 178	0/15
	7.5	181, 211	0/16
	9	222	3/8

[a] 10^6 cells/mouse iv in CD2F1. Treatment iv on days 1, 4, 7 after tumor transplantation (day 0). [b] Median survival time of treated mice/median survival time of controls × 100. [c] Number of toxic deaths/total number of mice. [d] Maleate salt.

using the P388 murine leukemia model, and the results are tabulated in Table 3 along with comparative data for ametantrone (1a), mitoxantrone (1b) and doxorubicin.

A more in-depth antitumor study against P388 murine leukemia for 3c along with comparative data for mitoxantrone (1b) and doxorubicin is presented in Table 4.

Discussion

The data in Table 1 indicate several important structural features of the side chains in relation to the cytotoxic activity. A comparison of the methylene side arm variation from two (3c) to three (3g) to four (3h) indicates a progressive decrease of cytotoxicity as the number of methylene groups separating the ring and distal primary amino nitrogen increases. This trend is similar to that seen in the carbocyclic series.[1a,b] Chemotypes 3c and 3g exhibit cytotoxicities comparable to ametantrone (1a). The importance of the basic amino distal nitrogen can be seen in the comparison of 3c with the bis-amide 3d (inactive). The cytotoxicity data for secondary amino substitution reveals that little change occurs for 3m–o as the group changes from ethyl, *n*-propyl, and isopropyl, respectively. The importance of a free terminal hydroxyl group for the cytotoxicity is indicated in the comparison of 3f and 3j. The azaametantrone analogue 3f shows a potency similar to that found for ametantrone (1a).

The trends exhibited by the tertiary amines 3a,b and 3p–t are difficult to rationalize and may reflect subtle balances of steric and pK_a effects. The aziridino (3b) and dimethyl (3a) analgoues are particularly active *in vitro* against L1210 cells and exhibit cytotoxicities nearly approaching that of mitoxantrone (1b).

A comparison of the *in vitro* data for analogues 3 and mitoxantrone (1b) found in Table 2 shows that, in general, the cytotoxic potency of 3 is decreased in comparison to mitoxantrone (1b) in both the sensitive and the doxorubicin-resistant LoVo tumor cell line. In both cell lines, 3a and 3u are the most and least cytotoxic compounds, respectively. The cytotoxic activity of the tested analogues 3 depends on both the structural and the basic characteristics of the alkylamino side chains. The presence of a distal dimethylamino (3a) or methylamino (3l) connected to the tricyclic quinonoid system through an ethylene bridge provides the most cytotoxic compounds against the sensitive cell line. The cytotoxic potential of these derivatives is 2–5 times higher than that of the (2-aminoethyl)amino congener 3c.

With the exception of 3h on the resistant cell line, in both cell lines increasing the length of the methylene chain results in a progressive decrease of the cytotoxic potential as the number of carbons separating the ring and the distal basic nitrogen function increases (see the homologous series 3a, 3t and 3c,g,h).

Steric variations on the distal nitrogen (see the series 3c,p,q and 3l,n,o) also result in analogoues 3 with significantly reduced cytotoxic potency. On the other hand the trends seen in the tertiary amines 3a and 3p–s are difficult to rationalize.

The reduced cytotoxic potency of the azaametantrone congener 3f in comparison with both ametantrone (1a) and mitoxantrone (1b) is worthy of notice and may be due to changes in hydrophobicity. It also demonstrates that SAR studies done with the carbocyclics cannot be assumed to be predictive for the heterocyclic congeners.

The lack of cross resistance with doxorubicin in the LoVo/Dx tumor cell line is a noteworthy peculiarity of all the analogues of 3 carrying a distal tertiary amine in the side chain, suggesting that these derivatives are able to overcome the MDR in the LoVo/DX cell line.

Oxidation of the nuclear nitrogen atom of 3c and 3a to give the related *N*-oxides 4a and 4b, respectively, results in a marked decrease (10–30-fold) of the cytotoxic potency against both LoVo cell lines. Interestingly, in this series the tertiary amino-substituted derivative 4b was endowed with partial cross resistance with doxorubicin.

Among the analogues 3 tested, significant *in vivo* antitumor activity against the P388 murine leukemia is observed with 3a and its 2-amino and 2-methylamino congeners (3c and 3l, Table 3). At the highest tolerated doses, these three compounds show an antileukemic activity comparable or superior (3c) to that of mitoxantrone (1b).

Of note is the fact that 3c is active (%T/C 162–230) over a wide range of well-tolerated doses (8–27 mg/kg), as shown in Table 4. This compound appears to be endowed

with a marked reduction of potency (about 10 times) with respect to mitoxantrone (**1b**), a feature which is also shared by **3a**. Only **3l** shows an antitumor potency close to those of mitoxantrone (**1b**).

The α,α-dimethyl derivative of **3c**, namely **3i**, which was synthesized in an attempt to avoid a possible metabolic inactivation of **3c** triggered by α-oxidation of the (2-aminoethyl)amino side chain, is devoid of any significant *in vivo* antileukemic activity.

As was observed in the *in vitro* studies, a progressive decrease of the antileukemic activity results as the number of carbon atoms separating the ring and the distal basic nitrogen function increases. Accordingly, the (3-aminopropyl)amino derivative **3g** retains antitumor activity, although at higher dosages (%T/C 155 at the highest tested dose of 40 mg/kg) while the (4-aminobutyl)amino analogue **3h** is completely inactive at the highest tested dose of 35 mg/kg. A substantial decrease in activity at the highest tested doses is found for **3p**, **3r**, and **3s** in comparison to analogue **3a**.

Surprisingly, the azaametantrone congener **3f** is only marginally active at the highest tested dose of 40 mg/kg. The pronounced antitumor activity of **3c** is retained in the related *N*-oxide **4a**, although the compound appears to be less potent than **3c**. Given that **4a** displayed little *in vitro* activity but significant *in vivo* activity suggests that the *N*-oxide may undergo bioactivation in the whole animal and may serve as a prodrug for the related **3c**. On the other hand only marginal activity at the highest tested doses was observed with **4b**, the *N*-oxide related to **3a**.

Conclusions

We have developed synthetic strategies which lead to several congeners **3** and two related *N*-oxides **4** which exhibit a wide range of *in vitro* and *in vivo* antitumor activity. The introduction of a nitrogen functionality into the 2-position of the anthracenedione chromophore to give the 6,9-bis((aminoalkyl)amino)benzo[*g*]isoquinoline-5,10-diones **3** and the related *N*-oxides **4** exerts a substantial effect on the biological activity of the molecules. This modification results in specific analogues of **3** endowed with significant antileukemic activity in mice. In particular compound **3c** is capable of antileukemic activity superior to that of mitoxantrone, in a wide range of well-tolerated dosages.

Another effect of this nitrogen for carbon isosteric replacement at the 2-position can be seen by the surprising loss of *in vitro* and *in vivo* activity observed with the 2-azaametantrone congener **3f**. This observation is of note as it demonstrates that caution must be used when attempting to predict the SAR of a new class of compounds based upon information obtained from an established series of compounds, regardless of the seemingly apparent similarities of the two classes.

It is interesting that when the nitrogen functionality is introduced in the 1-position, compounds with a significantly reduced activity against murine leukemia models are obtained, as is the case for 1-aza analogue related to **3c**.[17a] This suggests that in the bioisosteres the positioning of the nitrogen functionality is critically important in the expressed antitumor activity. The effect of the aza substitution on DNA affinity and drug-induced DNA damages of 2-aza- and 1-azaanthracenediones is currently being investigated.[24]

Compound **3c** (6,9-bis[(2-aminoethyl)amino]benzo[*g*]-isoquinoline-5,10-dione dimaleate; BBR 2778) appears to be the most promising second-generation anthracenedione analogue so far developed.

Besides its activity against the P 388 murine leukemia, BBR 2778 is more effective *in vivo* and is endowed with a better therapeutic index than mitoxantrone (**1b**) on a panel of murine hematological tumors and lymphomas (ascitic L 1210 leukemia; YC-8 lymphoma).[25] Its efficacy is comparable to that of mitoxantrone (**1b**) against murine (3LL lung carcinoma; B16 melanoma) and human (MX-1 mammary carcinoma) solid tumors.[25] Preliminary tolerability studies indicate that BBR 2778 is not cardiotoxic and, at equiactive doses, less leukopenic than mitoxantrone,[26] suggesting that it is a promising candidate toward clinical development.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Proton and carbon NMR spectra were run on a Bruker WP-270SY or WM-250 pulsed Fourier transform spectrometer. For thin-layer chromatography, precoated silica gel or alumina plates (Eastman Chromagram sheets) with fluorescent indicator were used. 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, or by Redox s.n.c., Cologno Monzese, Milan, Italy. Where analyses are indicated only by elemental symbols, results were within ±0.4%.

Biological Studies. *In Vitro* Cytotoxicity Evaluations: L1210 Murine Leukemia. L1210 murine leukemia cells were routinely maintained as suspension cultures in McCoy's 5A medium supplemented with 10% horse serum and grown in a humidified environment of 10% carbon dioxde and 90% air at 37 °C. To assess the *in vitro* toxicity, each compound was dissolved in water and added to 1 mL of L1210 cells (10^5 cells/tube) to attain final concentrations of 0.01, 0.1, 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 using the following formula:

$$\%\ \text{growth inhibition} = 1 - [\text{cell number treated/cell number control}] \times 100$$

The growth inhibition data was then used to calculate the IC_{50} vlaue (the calculated drug concentration required to inhibit cell growth by 50% of control).

Human Colon Adenocarcinoma LoVo and LoVo/Dx. LoVo and LoVo/Dx were cultured in Ham's F12 medium (Gibco) supplemented with 10% fetal calf serum, 1% L-glutamine, 200 mM, 1% BME vitamins solution 100X, 2% Hepes buffer solution 1 M in 0.85% NaCl, and 1% penicillin 5000 UI/mL–streptomycin 5000 μg/mL solution.

Cells were split two times a week and maintained at 37 °C in an atmosphere of CO_2. The cell lines were periodically tested for Mycoplasma contamination with Dapi test (Boehringer Mannheim). For the cytotoxicity evaluation of the compounds the MTT colorimetric assay[27] was used. Briefly, MTT assay is based on mitochondrial reduction of tetrazolium salt by living cells. The viable cell number is proportional to the production of formazan salts which can be read spectrophotometrically at 570 nm.

A total of 2.5×10^5 cells/mL for each cell line were plated in 96-microwell plates (Nunclon Delta, Nunc, Roskilde, Denmark) and preincubated for 24 h. After this time the tumor cell lines were exposed to drugs dissolved in appropriate solvent for 144 h. The drug concentration inhibiting 50% of cellular growth (IC_{50}, μg/mL) and the resistance index (RI: IC_{50} of resistant cell line/IC_{50} of sensitive cell line) was calculated.

***In Vivo* Biological Studies: P388 Murine Leukemia.** P388 murine leukemia cells were maintained *in vivo* by serial intraperitoneal (ip) injections of 10^5 cells in DBA2 male mice. For test purposes, CD2F1 mice were inoculated intravenously (iv) with 10^6 P388 cells, and treatment was initiated 24 h later. The iv dose of drug was administered on days 1, 4, and 7 after tumor

transplantation. Mice were observed daily for signs of toxicity and survival. The date of death was recorded for each animal during the 60-day study. The median survival time (MST) for each treatment group was calculated and the % T/C was determined using the following formula:

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

Synthesis: 6,9-Bis[[2-(dimethylamino)ethyl]amino]benzo[*g*]isoquinoline-5,10-dione (3a). (a) General Procedure A from 6a (*in Situ* Formation of 7). A solution of **6a** (0.110 g, 0.46 mmol), anhydrous sodium carbonate (23 mg, 0.22 mmol), and sodium dithionite (68 mg, 0.39 mmol) in dry ethanol (12 mL) was refluxed for 1 h. *N,N*-Dimethylethylenediamine (1.61 g, 18.2 mmol) was added, and the mixture was refluxed for 18 h. The solvent and excess amine were evaporated under reduced pressure and the residue chromatographed on silica gel. A major blue band was eluted with 1:1 methanol–chloroform to yield **3a** (0.066 g, 38%). Recrystallization from dichloromethane and high-boiling ligroine afforded blue needles: mp 164–166 °C; ^{1}H NMR ($CDCl_3$) δ 11.06 (br t, 1H), 10.97 (br t, 1H), 9.63 (s, 1H), 8.92 (d, 1H), 8.13 (d, 1H), 7.32 (m, 2H), 3.54 (q, 4H), 2.70 (t, 4H), 2.38 (s, 12H); mass spectrum, *m/z* (relative intensity) 381 (2.0, M^+), 59 (5.4), 59 (100); UV λ_{max} nm (ϵ) [2-methoxyethanol] 580 (sh, 6200), 614 (10 600), 661 (12 700). Anal. ($C_{21}H_{27}N_5O_2$) C, H, N.

(b) General Procedure B from Difluoride 6b. A mixture of **6b** (219 mg, 0.893 mmol) and *N,N*-dimethylethylenediamine (1 mL) in pyridine (1 mL) was stirred at room temperature for 48 h. Most of the pyridine was removed under a slow stream of nitrogen, and the residue was placed under vacuum. The solid was chromatographed over silica gel using gradient elution of chloroform–methanol mixtures from 0% methanol to 50% methanol. The eluents were removed from the eluted blue band to yield **3a** (278 mg, 82%).

6,9-Bis[[2-(1-aziridino)ethyl]amino]benzo[*g*]isoquinoline-5,10-dione (3b). A similar reaction using procedure A with **6a** (0.20 g, 0.83 mmol) and *N*-(2-aminoethyl)aziridine (0.71 g, 8.21 mmol) at reflux for 18 h followed by chromatography (1:9 methanol–chloroform) gave **3b**. Recrystallization from a mixture of methylene chloride and ligroine yielded a dark blue solid (17 mg, 5%): mp 100–103 °C; ^{1}H NMR ($CDCl_3$) δ 11.23 (br, 1H), 11.15 (br, 1H), 9.73 (s, 1H), 8.91 (d, 1H), 8.12 (d, 1H), 7.40 (d, 1H), 7.38 (d, 1H), 3.69 (q, 4H), 2.60 (t, 4H), 1.85 (m, 4H), 1.24 (m, 4H); mass spectrum, *m/z* (relative intensity) 377 (100, M^+), 278 (72.7), 56 (12.6). Anal. ($C_{21}H_{23}N_5O_2$) C, H, N.

6,9-Bis[(2-aminoethyl)amino]benzo[*g*]isoquinoline-5,10-dione (3c). (a) A similar reaction using procedure A with **6a** (0.40 g, 1.7 mmol) and ethylenediamine (1.16 g, 20.3 mmol) at reflux for 1 h followed by chromatography (1:1 methanol–chloroform) led to several reddish-purple fractions which on ^{1}H NMR analysis could be identified as **8a** and **8b**. A major blue fraction eluted with 1:5:5 triethylamine–methanol–chloroform which was identified as **3c**: ^{1}H NMR ($CDCl_3$) δ 11.23 (br m, 1H), 11.15 (br m, 1H), 9.63 (s, 1H), 8.92 (d, 1H), 8.11 (d, 1H), 7.34 (d, 1H), 7.32 (d, 1H), 3.54 (m, 4H), 3.10 (t, 4H); mass spectrum, *m/z* (relative intensity) 325 (100, M^+), 278 (71.0), 266 (53.6). All attempts to crystallize this sample resulted in decomposition.

Hydrochloride Salt: (1) From Amine 3c. Hydrogen chloride gas was bubbled through a solution of the amine in chloroform. The solid was collected by filtration and dried to yield a dark blue hygroscopic solid (0.174 g, 26%): mp 209–212 °C; ^{1}H NMR (DMSO-d_6) δ 10.91 (br, 2H), 9.44 (s, 1H), 9.01 (d, 1H), 8.23 (br, 4H), 8.09 (d, 1H), 7.74 (br s, 2H), 3.84 (m, 4H), 3.02 (m, 4H). The free amine could be regenerated by adding solid potassium carbonate to the NMR sample. Analysis by ^{1}H NMR indicated the presence of the free amine.

(2) From Deprotection of t-Boc Analogue 3e. Hydrogen chloride gas was bubbled through a solution of **3e** (0.160 g, 0.30 mmol) in dry chloroform (10 mL) for 30 min. The dark blue solid was filtered and dried (0.115 g, 95%), mp 213–215 °C whose structure was confirmed as the hydrochloride salt of **3c** by ^{1}H NMR analysis.

***N*-Bis-acetyl Derivative 3d.** Procedure A was repeated, and the free amine was acetylated to obtain a derivative for microanalysis. The crude reaction mixture was applied to a silica gel column. Acetic anhydride (30 mL) was added directly to the column, and it was allowed to stand for 15 min. A major blue fraction of **3d** eluted with 1:4 methanol–chloroform, which was then crystallized from a methanol–chloroform mixture to yield a blue solid (0.240 g, 35%): mp 155–156 °C; ^{1}H NMR (DMSO-d_6) δ 11.12 (t, 1H), 11.02 (t, 1H), 9.42 (s, 1H), 8.95 (d, 1H), 8.15 (br, 2H), 8.03 (d, 1H), 7.62 (br s, 2H), 3.57 (m, 8H), 1.83 (s, 6H); mass spectrum, *m/z* (relative intensity) 409 (2.5, M^+), 337 (6.4), 86 (100). Anal. ($C_{21}H_{23}N_5O_2$) C, H, N.

(c) From Difluoride 6b. A suspension of **6b** (4.00 g, 16.31 mmol) in dry pyridine (80 mL) was heated to 40 °C under a nitrogen atmosphere until the solid dissolved. The solution was cooled to room temperature, and ethylenediamine (8.72 mL, 130.5 mmol) was added in one portion. The reaction mixture was stirred mechanically at room temperature for 20 h and then for 2 h at 50 °C. After cooling to room temperature, the mixture was kept at −5 °C for 0.5 h and then filtered. The solid was washed with pyridine (5 mL) and dried under vacuum at 30 °C overnight. This product was obtained as a blue solid (6.5 g), and elemental analysis indicated the presence of fluorine. This crude material was used to form the dimaleate salt without further purification: HPLC Lichrospher 100 RP18 (5 μm, 150 mm); eluant, sodium heptanesulfonate 20 mM in water/acetonitrile/dioxane (72/20/5), pH 3, by phosphoric acid; flow 1 mL/min; λ 280 nm; 96.5% (area); retention time, 8.22 min; ^{1}H NMR ($CDCl_3$) δ 11.19 (m, 2H, exchangeable with D_2O), 9.63 (d, 1H), 8.94 (d, 1H), 8.12 (dd, 1H), 7.35 (m, 2H), 3.53 (q, 4H), 3.10 (t, 4H).

Maleate Salt. The crude product from above (5.44 g) was dissolved in ethanol/methanol, 85/15 (310 mL), at 40 °C under a nitrogen atmosphere, and then a solution of maleic acid (4.509 g, 38.46 mmol) in ethanol (46 mL) was added rapidly. The mixture was stirred for 0.25 h at 40 °C, allowed to cool to room temperature, and then placed in the refrigerator at −4 °C for 2 h. The solid was collected by filtration, washed with ethanol and then with ether, and dried under vacuum. The crude product (9.42 g) was suspended in distilled water (95 mL) at 50 °C, and ethanol (220 mL) was added until complete dissolution. Additional ethanol (440 mL) was added, and the mixture was stirred at room temperature overnight. The solid was collected by filtration, washed with ethanol and ether, and dried under vacuum. The product **3c** dimaleate was obtained as a blue solid (6.80 g, 85% overall yield from crude **3c**): mp 192 °C dec (DSC); TGA, 4.17% weight loss (35–150 °C) corresponding to 1.3 mol of water; UV λ_{max} nm (ϵ) [water] 246 (26 130), 273 (13 933), 313 (5354), 597 (13 242), 641 (14 607); HPLC Lichrospher 100 RP18 (5 μm, 150 mm); eluent, sodium heptanesulfonate 20 mM in water/acetonitrile/dioxane (75/20/5), pH 3, by phosphoric acid; flow 1 mL/min; λ 280 nm; 96.1% (area); retention time 8.28 min; ^{1}H NMR (D_2O) 8.96 (s, 1H), 8.73 (d, 1H), 7.72 (d, 1H), 7.10 (s, 2H), 6.17 (s, 4H), 3.80 (m, 4H), 3.34 (m, 4H). Anal. ($C_{25}H_{27}N_5O_{10}\cdot 1.3H_2O$) C, H, N.

6,9-Bis[[2-[*N*-(*tert*-butoxycarbonyl)amino]ethyl]amino]benzo[*g*]isoquinoline-5,10-dione (3e). (a) Via procedure A, **6a** (0.25 g, 1.0 mmol) and *N*-(*tert*-butoxycarbonyl)ethylenediamine (1.5 g, 9.4 mmol) were refluxed for 18 h. The ethanol was evaporated, water (20 mL) was added to the residue, and the blue solid was collected by filtration. Chromatography (5% methanol in chloroform) gave a major blue fraction which on crystallization from a chloroform–carbon tetrachloride mixture led to **3e** (0.265 g, 48%): mp 215–216 °C; ^{1}H NMR ($CDCl_3$) δ 11.07 (br, 1H), 10.96 (br, 1H), 9.49 (s, 1H), 8.90 (d, 1H), 7.99 (d, 1H), 7.32 (br s, 2H), 5.30 (br, 2H), 3.58 (m, 4H), 3.47 (m, 4H), 1.56 (s, 18H); mass spectrum *m/z* (relative intensity) 525 (100, M^+) 339 (51.9), 57 (87.7). Anal. ($C_{27}H_{35}N_5O_6$) C, H, N.

(b) Via procedure B, **6b** (0.10 g, 0.41 mmol) and *N*-(*tert*-butoxycarbonyl)ethylenediamine (0.65 g, 4.10 mmol) in pyridine (4.0 mL) were stirred for 48 h. Addition of water (20 mL) led to **3e** which was collected by filtration (0.17 g, 80%), mp 213–216 °C.

6,9-Bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]benzo[*g*]isoquinoline-5,10-dione (3f): From Dimesylate 10b. A solution of **10b** (0.40 g, 0.83 mmol) and 2-(trimethylsiloxy)ethylamine[23] (2.21 g, 16.5 mmol) in pyridine (5.0 mL) was stirred at room temperature under a nitrogen atmosphere for 48 h. The pyridine was removed under a nitrogen flow, and the residue was taken up in methylene chloride. This solution was washed with saturated sodium bicarbonate and dried over sodium sulfate. Removal of the solvent left a blue oil which was dried under vacuum overnight. Chromatography on a silica gel column

resulted in a cleavage of the Si–O bond to yield **3f**. This product was eluted with a 1:5:5 triethylamine–methanol–chloroform mixture. Concentration gave **3f** as an oil: ^{1}H NMR ($CDCl_3$) δ 11.19 (br, 1H), 11.06 (br, 1H), 9.46 (s, 1H), 8.83 (d, 1H), 7.98 (d, 1H), 6.98 (s, 2H), 3.82 (m, 4H), 3.52 (m, 4H), 3.07 (m, 4H), 2.96 (m, 4H).

From 6b. A solution of 2-[(2-aminoethyl)amino]ethanol (20.6 mL, 0.204 mol) in dry pyridine (20 mL) was added over a 0.5-h period to a mechanically stirred suspension of **6b** (5.00 g, 20.4 mmol) in dry pyridine (70 mL) at 0 °C under a nitrogen atmosphere. The mixture was stirred for 1.5 h at 0 °C and at room temperature for 3 h. While keeping the water bath at room temperature, the mixture was concentrated to half volume with a rotary evaporator. The residue was diluted with dichloromethane (200 mL) and stored overnight in a refrigerator at −20 °C. After removal of the dichloromethane by distillation at reduced pressure and room temperature, the residue was applied to a chromatographic column (silica gel, 3000 g) and eluted with chloroform–methanol–concentrated ammonium hydroxide mixtures from 100:0:0 to 80:20:1. The fractions containing significant amounts of product were combined and concentrated on a rotary evaporator while keeping the water bath at 30 °C. The oily residue contained 4 mol of 2-[(2-aminoethyl)amino]ethanol per mole of product (^{1}H NMR analysis): it was further purified by column chromatography (silica gel, 280 g, eluent chloroform–methanol–concentrated ammonium hydroxide from 100:0:0 to 70:30:2). The fractions containing product were combined and concentrated on a rotary evaporator, keeping the water bath at 30 °C. The resulting blue oil which still contained some starting diamine was taken up in ethanol (40 mL), the solution was concentrated, the residue was taken up in dichloromethane (100 mL) and stored overnight in a refrigerator at −20 °C. When the solution was allowed to warm to room temperature, the product began to crystallize; then it was concentrated to half volume with a rotary evaporator, keeping the water bath at room temperature. The mixture was placed in a refrigerator at 4 °C for 2 h and at −7 °C overnight. The blue crystals were collected by filtration under nitrogen and dried under high vacuum at room temperature. This material (1.08 g) had a HPLC purity of 93.3% (area). The crude product was dissolved in a mixture of absolute ethanol (3 mL) and dichloromethane (10 mL), and while the mixture was heated at reflux under a nitrogen atmosphere, additional dichloromethane (40 mL) was added over a period of 6–7 min. The solution was cooled to room tempeature and stored in a refrigerator overnight. After warming to room temperature, *tert*-butyl methyl ether (20 mL) was added to the resulting suspension which was then kept at room temperature for 30 min and at 4 °C for 2 h. The blue crystals were collected by filtration under nitrogen and dried under high vacuum at room temperature to yield **3f** (973 mg, 11.5% yield): mp 132–134 °C; ^{1}H NMR ($CDCl_3$) δ 11.22 (m, 1H, exchangeable with D_2O), 11.13 (m, 1H, exchangeable with D_2O), 9.52 (s, 1H), 8.87 (d, 1H), 8.05 (d, 1H), 7.10 (m, 2H), 3.73 (m, 4H), 3.55 (m, 4H), 3.05 (t, 4H), 2.93 (t, 4H).

Maleate Salt. A solution of maleic acid (600 mg, 5.17 mmol) in absolute ethanol (5 mL) was added to a stirred solution of **3f** (950 mg, 2.3 mmol) in a mixture of absolute ethanol (35 mL) and methanol (20 mL) at 40 °C under a nitrogen atmosphere. The reaction mixture was allowed to cool to room temperature, and stirring was stopped when crystals formed. After 2 h at room temperature, the mixture was cooled at 0 °C with an ice bath for 1 h. The blue crystals were collected by filtration under nitrogen, washed with absolute ethanol and ether, and dried under vacuum at room temperature. This crude material (1.34 g) was suspended in distilled water (4 mL), and absolute ethanol (10 mL) was added until complete dissolution. Additional ethanol (60 mL) was slowly added while stirring, and the crystallization was induced by scratching of the reaction vessel. The mixture was stored in a refrigerator at 4 °C overnight. The blue dimaleate crystals were collected by filtration, washed with absolute ethanol and ether, and dried under high vacuum at 40 °C for 2 h (1.17 g, 76% yield): mp 142 °C dec (DSC); TGA 3.31% weight loss (35–120 °C), corresponding to 1.2 mol of water: UV λ_{max} nm (ε) [water] 247 (28 256), 272 (16 103), 313 (6726), 597 (14 428), 642 (15 753); HPLC Lichrospher 100 RP18 (5 μm, 150 mm); eluent, sodium heptanesulfonate 20 mM in water–acetonitrile–dioxane (75/20/5), pH 3, by H_3PO_4; flow 1 mL/min; λ 280 nm; >99% (area); retention time 5.50 min; ^{1}H NMR (D_2O) δ 8.92 (s, 1H), 8.71 (d, 1H), 7.67 (d, 1H), 7.05 (s, 2H), 6.03 (s, 4H), 3.70–3.90 (m, 8H), 3.25–3.45 (m, 8H). Anal. ($C_{29}H_{35}N_5O_{12}\cdot 1.2H_2O$) C, H, N.

6,9-Bis[(3-aminopropyl)amino]benzo[*g*]isoquinoline-5,-10-dione (3g). A solution of 1,3-diaminopropane (870 mg, 11.76 mmol) in chloroform (3 mL) was added to **6b** (300 mg, 1.22 mmol) in chloroform (6 mL). The purple mixture was allowed to stir for 166 h at room temperature. The mixture was filtered and the salts washed with chloroform. The solvent was removed by rotary evaporation, and the residue was placed under vacuum overnight to yield 290 mg (93%) of the bis-amine [TLC, silica gel, 1:3 methanol–chloroform–few drops of aq ammonium hydroxide indicated one blue spot]: mp 105 °C (softens) 112–115 °C; ^{1}H NMR (DMSO-d_6) δ 11.15 (m, 1H), 11.05 (m, 1H), 9.45 (s, 1H), 8.90 (d, 1H), 8.0 (d, 1H), 7.55 (br s, 2H), 3.55 (br m, 4H), 4.65 (t, 4H), 3.75 (t, 4H), 1.75 (t, 4H).

The maleate salt was prepared by adding a solution of maleic acid (100 mg, 0.86 mmol) in methanol to **3g** (100 mg, 0.28 mmol) dissolved in methanol (2 mL) and ethyl acetate (2 mL). The addition of more ethyl acetate gave a blue precipitate which was collected by filtration and dried under vacuum (120 mg, 79%): ^{1}H NMR (DMSO-d_6) δ 11.10 (t, 1H), 11.00 (t, 1H), 9.45 (s, 1H), 8.95 (d, 1H), 8.05 (d, 1H), 7.75 (br, 4H), 7.60 (s, 2H), 6.0 (s, 4H), 3.60 (m, 4H), 2.95 (t, 4H), 1.95 (m, 4H). Anal. ($C_{27}H_{31}N_5O_{10}$) C, H, N.

6,9-Bis[(4-aminobutyl)amino]benzo[*g*]isoquinoline-5,10-dione (3h). A solution of 1,4-diaminobutane (960 mg, 10.9 mmol) in chloroform (3 mL) was added to **6b** (319 mg, 1.3 mmol) in chloroform (6 mL) and the mixture was stirred for 217 h. Workup as in **3g** gave **3h** (400 mg, 80%): TLC (1:3 methanol–chloroform–few drops of aqueous ammonium hydroxide); mp 80 °C (softens) 90–92 °C; ^{1}H NMR (DMSO-d_6) δ 11.15 (m, 1H), 11.05 (m, 1H), 9.42 (s, 1H), 8.90 (d, 1H), 8.0 (d, 1H), 7.5 (br s, 2H), 3.45 (m, 4H), 2.60 (m, 4H), 1.70 (m, 4H), 1.45 (m, 4H).

The maleate salt was prepared by treatment of a solution of maleic acid (116 mg, 1 mmol) in ethyl acetate (4 mL) with **3h** (124 mg, 0.40 mmol) in a methanol (6 mL)–ethyl acetate (8 mL) solution. A blue oil separated upon which the mixture was placed in the refrigerator overnight. The solvents were removed by decantation, and the remaining solid was washed well with ethyl acetate to yield a blue hygroscopic solid: 125 mg (63%); ^{1}H NMR (DMSO-d_6) δ 11.20 (m, 1H), 11.10 (m, 1H), 9.45 (s, 1H), 8.95 (d, 1H), 8.05 (d, 1H), 7.70 (br s, 2H), 7.6 (s, 2H), 6.00 (s, 4H), 3.55 (m, 4H), 2.85 (m, 4H), 1.70 (m, 8H). Anal. ($C_{29}H_{35}N_5O_{10}$) C, H, N.

6,9-Bis[(2-amino-2,2-dimethylethyl)amino]benzo[*g*]isoquinoline-5,10-dione (3i). Treatment of **6b** (204 mg, 0.83 mmol) and 1,2-diamino-2-methylpropane (804 mg, 9.1 mmol) according to procedure B for 72 h followed by chromatography (gradient elution from 20% to 30% methanol in chloroform) led to **3i** (92 mg, 31%): ^{1}H NMR ($CDCl_3$) δ 11.65 (t, 1H), 11.60 (t, 1H), 9.75 (s, 1H), 9.00 (d, 1H), 8.20 (d, 1H), 7.30 (m, 2H), 3.40 (d, 4H), 1.30 (s, 6H). Anal. ($C_{21}H_{27}N_5O_2$) C, H, N.

6,9-Bis[[2-[(2-methoxyethyl)amino]ethyl]amino]benzo[*g*]-isoquinoline-5,10-dione (3j). A solution of **10b** (0.15 g, 0.21 mmol) and 2-methoxyethylamine (0.47 g, 6.20 mmol) in pyridine (2.0 mL) was stirred at room temperature under a nitrogen blanket for 48 h. The pyridine and excess amine were removed under vacuum. The residue was dissolved in methylene chloride, washed with a saturated sodium bicarbonate solution, and dried over sodium sulfate. Upon removal of the solvent the residue was purified by column chromatography on silica gel by eluting with 1:1 methanol–chloroform. Recrystallization from a ethanol and pentane mixture led to **3j** (0.091 g, 66%): mp 174–175 °C; ^{1}H NMR ($CDCl_3$) δ 11.08 (br, 1H), 10.97 (br, 1H), 9.56 (s, 1H), 8.90 (d, 1H), 8.05 (d, 1H), 7.31 (s, 2H), 3.62 (m, 8H), 3.43 (s, 6H), 3.08 (t, 4H), 2.94 (t, 4H), 2.66 (br s, 2H); mass spectrum, *m/z* (relative intensity) 441 (100, M^+), 354 (57.4), 266 (50.5). Anal. ($C_{25}H_{31}N_5O_4$) C, H, N.

6,9-Bis[[2-[*N*-(*tert*-butoxycarbonyl)-*N*-methylamino]-ethyl]amino]benzo[*g*]isoquinoline-5,10-dione (3k). Dione **6b** (0.29 g, 1.18 mmol) was added to a stirred solution of *N*-(*tert*-butoxycarbonyl)-*N*-methylethylenediamine (0.824 g, 4.73 mmol) in dry pyridine (5 mL). The reaction mixture was stirred in a nitrogen atmosphere for 24 h at room temperature and then for an additional 8 h at 50 °C. The solvent was removed under reduced pressure, the blue residue was taken up in dichloromethane (50 mL), and this solution was washed with a 5%

sodium bicarbonate solution (2 × 30 mL) and water (30 mL). The combined organic extract was dried over sodium sulfate and the solvent evaporated under reduced pressure. The blue residue was purified by column chromatography over silica gel with the eluent dichloromethane–ethyl acetate–methanol (93:5:2) to yield **3k** (400 mg, 61%): mp 161.5–162.5 °C (after recrystallization from dichloromethane–hexane); ^{1}H NMR ($CDCl_3$) δ 10.95–11.23 (m, 2H, exchangeable with D_2O), 9.62 (s, 1H), 8.95 (d, 1H), 8.12 (d, 1H), 7.25–7.55 (m, 2H), 3.45–3.75 (m, 8H), 2.94 (s, 6H), 1.49 (s, 18H).

6,9-Bis[[2-(methylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione trihydrochloride (3l). Ethanolic hydrogen chloride (6.7 N, 2.0 mL) was added to a solution of **3k** (440 mg, 0.795 mmol) in chloroform (40 mL). The mixture was stirred at room temperature under a nitrogen atmosphere for 24 h. The dark green crystals were collected by filtration, washed with absolute ethanol, and dried at 40 °C under vacuum to give **3l** (945 mg, 94%): mp 188 °C dec; ^{1}H NMR (D_2O) δ 9.27 (s, 1H), 8.85 (d, 1H), 8.17 (d, 1H), 7.38 (m, 2H), 3.80–3.95 (m, 4H), 3.30–3.45 (m, 4H), 2.75 (s, 6H). Anal. ($C_{19}H_{28}Cl_3N_5O_2 \cdot 2H_2O$) H, N; C: calcd, 46.51; found, 45.75; Cl: calcd, 21.32; found, 23.60.

6,9-Bis[[2-(ethylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (3m). Treatment of *N*-ethylethylenediamine (400 mg, 4.5 mmol) and **6b** (98 mg, 0.40 mmol) in pyridine for 66 h as in procedure B led to crude product which was dissolved in chloroform. The extract was washed with water and the resultant material chromatographed (gradient elution 5% methanol–95% chloroform as the initial eluent, with gradual changes to 10%, and then 50% methanol in chloroform). The product was eluted with 50% methanol–48% chloroform–2% concentrated ammonium hydroxide to yield **3m** (32 mg, 21%): mp 101–102 °C; ^{1}H NMR δ ($CDCl_3$) δ 11.10 (m, 1H), 11.00 (m, 1H), 9.6 (s, 1H), 8.9 (d, 1H), 8.05 (d, 1H), 7.3 (m, 2H), 3.55 (m, 4H), 3.0 (t, 4H), 2.8 (q, 4H), 1.15 (t, 6H). Anal. ($C_{21}H_{27}N_5O_2$) C, H, N.

6,9-Bis[[2-(propylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (3n). Treatment of *N*-propylethylenediamine (403 mg, 4.0 mmol) and **6b** (98 mg, 0.40 mmol) in pyridine for 24 h as in **3m** led to **3n**, which was purified by chromatography (gradient elution with the initial eluent being 5% methanol–95% chloroform followed by gradually increasing the methanol quantities to 10%, 20%, 30%, 40%, and 50%). Compound **3n** was eluted with 60% methanol–40% chloroform containing some concentrated ammonium hydroxide. The removal of the eluates afforded product **3n** (50 mg, 30%): mp 105–106 °C; ^{1}H NMR ($CDCl_3$) δ 11.15 (m, 1H), 11.05 (m, 1H), 9.6 (s, 1H), 8.9 (d, 1H), 8.10 (d, 1H), 7.3 (m, 2H), 3.6 (m, 4H), 3.0 (t, 4H), 2.75 (t, 4H), 1.6 (m, 4H, H_2O peak superimposed), 0.95 (t, 6H). Anal. ($C_{23}H_{31}N_5O_2$) C, H, N.

6,9-Bis[[2-(isopropylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (3o). Using a similar procedure to that described for **3m**, a pyridine solution of *N*-isopropylethylenediamine (450 mg, 4.5 mmol) and **6b** (110 mg, 0.45 mmol) was stirred for 40 h. Workup followed by chromatography (elution with 1:1 methanol–chloroform; some concentrated ammonium hydroxide) led to **3o** (56 mg, 33%); mp 135–136 °C; ^{1}H NMR ($CDCl_3$) δ 11.10 (br t, 1H), 11.00 (br t, 1H), 9.60 (s, 1H), 8.90 (d, 1H), 8.10 (d, 1H), 7.30 (m, 2H), 3.60 (m, 4H), 3.01 (m, 4H), 2.90 (m, 2H), 1.10 (d, 6H). Anal. ($C_{23}H_{31}N_5O_2$) C, H, N.

6,9-Bis[[2-(diethylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (3p). Using the procedure described for **3m**, *N,N*-diethylethylenediamine (684 mg, 5.9 mmol) and **6b** (202 mg, 0.82 mmol) in pyridine was stirred for 48 h. Workup followed by chromatography gave **3p** (330 mg, 84%): mp 142–144 °C; ^{1}H NMR ($CDCl_3$) δ 11.10 (t, 1H), 11.00 (t, 1H), 9.60 (s, 1H), 8.92 (d, 1H), 8.10 (d, 1H), 7.25 (m, 2H), 3.50 (m, 4H), 2.82 (t, 4H), 2.35 (m, 8H), 1.10 (t, 12H). Anal. ($C_{25}H_{35}N_5O_2$) C, H, N.

6,9-Bis[[2-(diisopropylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione (3q). A mixture of *N,N*-diisopropylethylenediamine (588 mg, 4.1 mmol) and **6b** (100 mg, 0.41 mmol) in methanol (1 mL)–water (1 mL) was stirred at room temperature for 88 h and then poured into ice water. The blue precipitate was collected by filtration and purified by column chromatography (initial eluent was chloroform followed by 2% and then 20% methanol in chloroform) to give **3q** (163 mg, 81%): mp 134–136 °C; ^{1}H NMR ($CDCl_3$) δ 11.10 (t, 1H), 11.00 (t, 1H), 9.63 (s, 1H), 8.92 (d, 1H), 8.13 (d, 1H), 7.33 (m, 2H), 3.45 (q, 4H), 3.10 (m, 4H), 2.82 (t, 4H), 1.08 (d, 24H). Anal. ($C_{29}H_{43}N_5O_2$) C, H, N.

6,9-Bis[(2-pyrrolidinoethyl)amino]benzo[g]isoquinoline-5,10-dione (3r). Using the procedure described for **3m**, 1-(2-aminoethyl)pyrrolidine (690 mg, 6.0 mmol) and **6b** (202 mg, 0.82 mmol) in pyridine were stirred for 49 h. Workup followed by chromatography (5% methanol–chloroform) led to **3r** (260 mg, 73%): mp 141–142 °C; ^{1}H NMR ($CDCl_3$) δ 11.15 (t, 1H), 11.00 (t, 1H), 9.65 (s, 1H), 8.90 (d, 1H), 8.05 (d, 1H), 7.35 (m, 2H), 3.65 (m, 4H), 2.90 (m, 4H), 2.70 (m, 8H), 1.85 (m, 8H). Anal. ($C_{25}H_{31}N_5O_2$) C, H, N.

The maleate salt was prepared by addition of a solution of maleic acid (271 mg, 2.34 mmol) in ethanol (5 mL) to a stirred suspension of **3r** (450 mg, 1.04 mmol) in ethanol (20 mL). After stirring for 2 h, ether (25 mL) was slowly added and the precipitate was filtered and dried under vacuum at 40 °C to yield the hygroscopic maleate salt of **3r** (580 mg): mp 164–166 °C. Anal. ($C_{33}H_{39}N_5O_{10}$) C, H, N.

6,9-Bis[(2-morpholinoethyl)amino]benzo[g]isoquinoline-5,10-dione (3s). Using the procedure described for **3m**, 4-(2-aminoethyl)morpholine (220 mg, 1.7 mmol) and **6b** (102 mg, 0.42 mmol) in pyridine were stirred for 120 h. Workup followed by chromatography (1:3 methanol–chloroform) led to **3s** (140 mg, 72%): ^{1}H NMR ($CDCl_3$) δ 11.05 (m, 1H), 10.90 (m, 1H), 9.62 (s, 1H), 8.90 (d, 1H), 8.10 (d, 1H), 7.25 (m, 2H), 3.80 (m, 8H), 3.55 (m, 4H), 2.75 (t, 4H), 2.50 (m, 8H). Anal. ($C_{25}H_{31}N_5O_4$) C, H, N.

6,9-Bis[[3-(dimethylamino)propyl]amino]benzo[g]isoquinoline-5,10-dione (3t). Using the procedure described for **3m**, a pyridine solution of 3-(dimethylamino)propylamine (700 mg, 6.9 mmol) and **6b** (100 mg, 0.41 mmol) was stirred at room temperature for 120 h. Purification by chromatography (initial eluent was 10% methanol–90% chloroform followed by 25% methanol–75% chloroform and then addition of small amounts of concentrated ammonium hydroxide to the latter eluent) led to **3t** (92 mg, 55%): mp 107–109 °C; ^{1}H NMR ($CDCl_3$) δ 11.00–11.20 (2 t, 2H), 9.63 (s, 1H), 8.93 (d, 1H), 8.12 (d, 1H), 7.37 (s, 2H), 3.55 (q, 4H), 2.55 (m, 4H), 2.35 (s, 12H), 2.00 (m, 4H). Anal. ($C_{23}H_{31}N_5O_2$) C, H, N.

6,9-Bis[[2-(2-hydroxyethoxy)ethyl]amino]benzo[g]isoquinoline-5,10-dione (3u). Using the procedure described for **3m**, a pyridine solution of 2-(2-aminoethoxy)ethanol (446 mg, 4.25 mmol) and **6b** (100 mg, 0.41 mmol) was stirred at room temperature for 45 h. Removal of the chloroform led to **3u** (140 mg, 82%), mp 97–99 °C. This material on TLC analysis showed a blue spot on elution with 1:9 methanol–chloroform and trace amount of impurities. ^{1}H NMR ($CDCl_3$) δ 11.15 (t, 1H), 11.06 (t, 1H), 9.59 (s, 1H), 8.84 (d, 1H), 7.98 (d, 1H), 7.10 (m, 2H), 3.83 (m, 8H), 3.75 (m, 4H), 3.55 (m, 4H). Anal. ($C_{21}H_{25}N_3O_6$) C, H, N.

6,9-Bis[(2-aminoethyl)amino]benzo[g]isoquinoline-5,10-dione 2-Oxide Dimaleate (4a). A mixture of **11** (1.20 g, 4.59 mmol) and ethylenediamine (2.46 mL, 36.75 mmol) in dry pyridine (20 mL) was stirred under a nitrogen atmosphere at room temperature for 6 h and then at 55 °C for 2 h. The reaction mixture was kept at 4 °C for 16 h; the solid was collected by filtration, washed with dry pyridine (3 mL) and dichloromethane (4 mL), and dried under vacuum at 40 °C for 4 h. The compound **4a**, partially present as the hydrofluoride salt, was obtained as a blue solid (1.68 g, 107%, mp 164 °C) and was used in the subsequent step without further purification. Under a nitrogen atmosphere a solution of maleic acid (1.12 g, 9.64 mmol) in methanol (10 mL) was added to a stirred refluxing solution of crude **4a** (1.68 g, 4.59 mmol) in chlorofrom–methanol (1:1) (100 mL). The mixture was heated at reflux for a further 5 min and then allowed to cool to room temperature. The solid was collected by filtration, washed with ethanol and then with ether, and dried under vacuum. The crude product (2.045 g) was suspended in distilled water (25 mL) at 70 °C, and ethanol (10 mL) was added until complete dissolution was obtained. Additional ethanol (290 mL) was added, and the mixture was allowed to cool to room temperature and kept at this temperature for 3 h and then at 4 °C overnight. HPLC purity of this product (1.70 g, 65% overall yield from **11**) was not satisfactory (89%), and it was recrystallized twice by the procedure described above. The product was obtained as small blue crystals (1.42 g, 51% overall yield from

11): mp (DSC) 190 °C dec: TGA 5.63% weight loss (35–150 °C), corresponding to 2 mol of water; UV λ_{max} nm (ϵ) [water] 213 (40 284), 215 (40 173), 275 (29 414), 613 (14 214), 658 (15 813); HPLC Lichrospher 100 RP18 (5 μm); eluent, sodium heptanesulfonate 10 mM + KH_2PO_4 10 mM in H_2O/CH_3CN/dioxane (75/20/5), pH 3, by H_3PO_4; flow 1 mL/min; λ 275 nm; >96% (area); retention time 4.16 min; ^1H NMR (D_2O) δ 8.65 (d, 1H), 8.39 (dd, 1H), 7.96 (d, 1H), 7.35 (s, 2H), 6.12 (s, 4H), 3.85 (q, 4H), 3.33 (m, 4H). Anal. ($C_{25}H_{27}N_5O_{11}\cdot 2H_2O$) C, H, N.

6,9-Bis[[2-(dimethylamino)ethyl]amino]benzo[g]isoquinoline-5,10-dione 2-Oxide (4b). *N,N*-Dimethylethylenediamine (0.33 mL, 3.02 mmol) was added to a stirred suspension of 11 (200 mg, 0.766 mmol) in dry pyridine (3 mL) under a nitrogen atmosphere at 67 °C. The mixture was stirred for 5 h at this temperature and overnight at room temperature. The reaction mixture was then applied to a silica gel column, eluting first with ethyl acetate, then with ethyl acetate–methanol (4:1), and finally with chloroform–methanol–concentrated ammonium hydroxide (16:4:0.2). The combined fractions coming from the major blue band were concentrated to dryness. The residue was triturated in ether (10 mL), and the solid was collected by filtration and dried under vacuum. The product **4b** was obtained as a blue solid (150 mg, 49%): mp 178 °C; UV λ_{max} nm (ϵ) [water] 278 (25 974), 368 (4465), 621 (12 617), 661 (13 643); HPLC Lichrospher 100 RP18 (5 μm); eluent sodium heptanesulfonate 10 mM + KH_2PO_4 10 mM in H_2O/CH_3CN/dioxane (70/20/10), pH 3, by H_3PO_4; flow 1 mL/min; λ 275 nm; > 97% (area); retention time 2.85 min; ^1H NMR ($CDCl_3$) δ 11.10 (m, 2H, exchangeable with D_2O), 9.01 (d, 1H), 8.35 (dd, 1H), 8.17 (d, 1H), 7.35 (s, 2H), 3.55 (q, 4H), 2.70 (t, 4H), 2.40 (s, 12H). Anal. ($C_{21}H_{27}N_5O_3$) C, H, N.

Pyridine-3,4-dicarboxylic Anhydride (5). A mixture of pyridine-3,4-dicarboxylic acid (15.0 g, 0.09 mol) and acetic anhydride (30 mL) was refluxed for 2 h. The excess acetic anhydride was removed by distillation, and the anhydride was purified by sublimation (123 °C at 3 mmHg) to yield **5** as a white solid (10.1 g, 76%): mp 74–76 °C (lit.[23] mp 75–76 °C); ^1H NMR δ ($CDCl_3$) 9.39 (s, 1H), 9.24 (d, 1H), 7.94 (d, 1H).

6,9-Dihydroxybenzo[g]isoquinoline-5,10-dione (6a). An intimately ground mixture of **5** (4.81 g, 0.032 mol) and 1,4-dimethoxybenzene (3.08 g, 0.022 mol) was added portionwise over a 3-min interval to a melt of aluminum chloride (60 g) and sodium chloride (12 g) held at 180 °C. This temperature was maintained for an additional 5 min. The hot mixture was cautiously decomposed with cold water (250 mL) and concentrated sulfuric acid (9 mL) while being cooled in an ice bath. The mixture was continuously extracted with chloroform and the product **6a** crystallized from ethanol as orange plates (1.08 g, 20%): mp 205–207 °C (lit.[18] mp 209 °C); ^1H NMR ($CDCl_3$) δ 12.85 (s, 1H), 12.68 (s, 1H), 9.64 (s, 1H), 9.15 (d, 1H), 8.14 (d, 1H), 7.40 (m, 2H).

6,9-Difluorobenzo[g]isoquinoline-5,10-dione (6b). The keto acid mixture of **9a** and **9b** (3.0 g, 0.011 mol) in fuming sulfuric acid (7.5 mL, 30% SO_3) was heated in an oil bath at 135–140 °C for 3 h. After cooling to room temperature, the mixture was poured over ice (200 mL) and neutralized with sodium bicarbonate. Extraction with methylene chloride gave **6b** as a yellow solid (2.0 g, 72%): mp 199–200 °C; ^1H NMR ($CDCl_3$) δ 9.54 (s, 1H), 9.12 (d, 1H), 8.03 (d, 1H), 7.57 (m, 2H). Anal. ($C_{13}H_5F_2NO_2$) C, H, N.

***leuco*-2-Azaquinizarin (7).** A mixutre of **6a** (0.40 g, 1.66 mmol) and NaOH (0.20 g, 5 mmol) in water was stirred at 70 °C until it became homogeneous. Sodium dithionite (0.74 g, 3.1 mmol) was added and the mixture stirred at 70 °C for 1 h. The mixture was cooled to 10 °C and filtered, and the residue was washed with water and dried under vacuum overnight to yield a brown solid (0.35, 87%): ^1H NMR ($CDCl_3$) δ 13.56 (s, 1H), 13.24 (s, 1H), 9.81 (s, 1H), 8.89 (d, 1H), 8.20 (d, 1H), 3.12 (s, 4H); ^{13}C NMR δ 201.5, 200.7, 155.6, 153.1, 149.3, 148.6, 133.6, 123.2, 116.6, 110.3, 108.9, 36.1, 35.8; mass spectrum, *m/z* (relative intensity) 243 (71.0, M^+).

4-(2′,5′-Difluorobenzoyl)nicotinic Acid (9a) and 3-(2′,5′-Difluorobenzoyl)isonicotinic Acid (9b). A. Via Friedel–Crafts Acylation. A mixture of **5** (5.0 g, 0.033 mol) and aluminum chloride (17.5 g, 0.131 mol) in 1,4-difluorobenzene (65 mL) was refluxed for 22 h. The excess 1,4-difluorobenzene was recovered by distillation. The residue was cooled in an ice bath and quenched with ice water (75 mL) and concentrated hydrochloric acid (6.3 mL). The precipitate solid was filtered and dried to yield a white powder (7.7 g, 87%). This material could be crystallized from acetonitrile and water: mp 214–217 °C; ^1H NMR (DMSO-d_6) δ 9.15 (s), 8.90 (d), 8.80 (d), 7.90 (d), 7.5 (m), 7.4 (m). Anal. ($C_{13}H_7F_2NO_3$) C, H, N.

B. Via Directed Metalation. *sec*-Butyllithium (1.32 M in cyclohexane; 1.35 mL, 1.79 mmol) was added dropwise via a syringe to a stirred solution of 1,4-difluorobenzene (203 mg, 1.78 mmol) in tetrahydrofuran (10 mL) at −76 °C which was kept under a nitrogen atmosphere. After being stirred for an additional 0.5 h, the yellow mixture was transferred dropwise via a jacketed cannula into a stirred solution of **5** (237 mg, 1.60 mmol) in tetrahydrofuran (35 mL) at −76 °C under a nitrogen blanket. The yellow solution was allowed to warm to room temperature and stirred for 15 h. The tetrahydrofuran was removed by rotary evaporation, and the resulting yellow residue was taken up in water (5 mL) and stirred in an ice bath. The solution was acidified with concentrated HCl, and the resulting precipitate was collected by filtration and washed with ice water (10 mL) and ether (2 mL). Recrystallization from acetonitrile gave a regioisomeric mixture as a white solid (312 mg, 75%): mp 217–220 °C; ^1H NMR (DMSO-d_6) δ 13.84 (br, 1H), 9.17 (s, 1H), 8.89 (d, 1H), 7.82 (m, 3H), 7.47 (m, 1H).

Methyl 4-(2′,5′-Difluorobenzoyl)nicotinate and Methyl 3-(2′,5′-difluorobenzoyl)isonicotinate. Ethereal diazomethane was added dropwise to a mixture of the keto acids **9a** and **9b** (12 mg, 0.05 mmol) in ether (0.5 mL) until effervescence ceased. The ether was evaporated, and the resulting solid was determined to be a 4:1 mixture of the regioisomeric esters derived from **9a** and **9b**, respectively, by ^1H NMR analysis (11 mg, 87%): mp 86–94 °C; major compound ^1H NMR ($CDCl_3$) δ 9.27 (s, 1H), 8.89 (d, 1H), 7.70 (m, 1H), 7.25 (m, 2H), 7.06 (m, 1H), 3.82 (s, 3H); minor compound ^1H NMR ($CDCl_3$) δ 8.99 (d, 1H), 8.71 (s, 1H), 7.85 (d, 1H), 7.70 (m, 1H), 7.25 (m, 1H), 7.00 (m, 1H, 3.78 (s, 3H).

6,9-Bis[(2-hydroxyethyl)amino]benzo[g]isoquinoline-5,-10-dione (10a). (a) Using procedure A, **6a** (0.50 g, 2.07 mmol) and 2-aminoethanol (8.10 g, 133 mmol) were refluxed for 18 h. The residue was poured into cold water (50 mL). The product was collected by filtration and recrystallized from methanol to yield dark blue needles (0.428 g, 63%): mp 237–239 °C; ^1H NMR (DMSO-d_6) δ 11.28 (t, 1H), 11.19 (t, 1H), 9.43 (s, 1H), 8.94 (d, 1H), 8.03 (d, 1H), 7.56 (s, 2H), 5.01 (t, 2H), 3.69 (m, 4H), 3.54 (m, 4H). Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

(b) From Difluoride 6b. Difluoride **6b** (1.0 g, 4.1 mmol) and 2-aminoethanol (2.5 g, 40.8 mmol) in pyridine (7 mL) were stirred at room temperature for 18 h. The mixture was poured into water (50 mL), and the product **10a** was filtered and dried (1.26 g, 94%), mp 236–239 °C.

6,9-Bis[[2-[(methylsulfonyl)oxy]ethyl]amino]benzo[g]-isoquinoline-5,10-dione (10b). A solution of **10a** (0.30 g, 0.92 mmol) in dry pyridine (4 mL) was stirred at room temperature under nitrogen for 10 min. Methanesulfonyl chloride (0.24 g, 2.07 mmol) was added and the mixture stirred for 20 min. The mixture was quenched into ice–water (20 mL), and the solid was filtered. Crystallization from a chloroform–ligroine mixture gave a blue solid (0.294 g, 66%): mp 116–118 °C; ^1H NMR ($CDCl_3$) 10.98 (br, 2H), 9.59 (s, 1H), 8.97 (d, 1H), 8.09 (d, 1H), 7.38 (d, 1H), 7.34 (d, 1H), 4.50 (t, 4H), 3.83 (q, 4H), 3.09 (s, 6H). Anal. ($C_{19}H_{21}N_3O_8S_2$) C, H, N.

6,9-Difluorobenzo[g]isoquinoline-5,10-dione 2-Oxide (11). To a solution of **6b** (2.03 g, 8.28 mmol) in dichloromethane was added portionwise *m*-chloroperoxybenzoic acid (3.90 g, assay 55%, 12.4 mmol) over a 2-min period. The mixture was stirred at room temperature for 24 h, diluted with dichloromethane (450 mL), washed with a 5% solution of sodium bicarbonate (3 × 50 mL), dried over sodium sulfate, and concentrated to dryness. The resulting residue was recrystallized from acetonitrile (150 mL). The product 11 was collected by filtration as orange-yellow crystals (1.544 g, 71%). An additional amount of pure 11 (200 mg, 9%) was obtained from the filtrate: mp 250–252 °C; ^1H NMR ($CDCl_3$) 8.82 (dd, 1H), 8.43 (dd, 1H), 8.11 (d, 1H), 7.58 (m, 2H). Anal. ($C_{13}H_5F_2NO_3$) C, H, N, F.

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