Synthesis of 4-Hydroxy-6,9-difluorobenz[*g*]isoquinoline-5,10-diones and Conversions to 4-Hydroxy-6,9-bis[(aminoalkyl)amino]-benz[*g*]isoquinoline-5,10-diones

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Received April 21, 1995
Revised August 31, 1995

Synthetic procedures have been developed which lead to 4-hydroxy-6,9-difluorobenz[*g*]isoquinoline-5,10-dione (**4a**) and its 3-methyl analogue **4b**. Attempts to displace the fluorides from **4a** with *N,N*-dimethylethylenediamine were unsuccessful. Analogue **4a** on treatment with *N*-(*t*-butoxycarbonyl)ethylene diamine led to **15**, formed from addition of the nucleophilic amine to C-3. On the other hand, analogue **4b**, on treatment with *N,N*-dimethylethylenediamine led to the anticipated difluoride displacement product **3c**. The protection of the hydroxy group of **4a** by benzylation with phenyldiazomethane led to **4c** which on treatment with *N*-(*t*-butoxycarbonyl)ethylene diamine or *N,N*-dimethylethylenediamine led to the corresponding 6,9-bis-substituted analogues **18a** and **18b**, respectively. Reductive debenzylations of **18a** and **18b** by hydrogenation over Pearlman's catalyst also effected partial reductions of the quinone. However, air oxidation of the over reduced products led to **3a** and **3b**, respectively. Treatment of **3a** with hydrogen chloride gas led to the hydrochloride salt of **3d**. Addition of *O*-*p*-Methoxybenzyl-*N,N'*-diisopropylurea to **4a** led to the *p*-methoxybenzyl analogue **4d**. Treatment of **4d** with *N,N*-dimethylethylene diamine or *N*-(*t*-butoxycarbonyl)ethylene diamine led to displacements of the fluorides to yield **18c** and **18d**, respectively. Deprotection of **18c** to **3b** was accomplished using methanesulfonic acid. Treatment of **18d** with trifluoroacetic acid followed by addition of maleic acid led to dimaleate salt of **3d**.

J. Heterocyclic Chem., **32**, 1693 (1995).

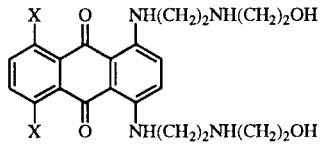
Introduction.

Anthracene-9,10-diones with specific 1,4-bis[(aminoalkyl)amino] substituents such as ametantrone (**1a**) and mitoxantrone (**1b**) (Figure 1) exhibit outstanding anticancer activities [1-6]. Mitoxantrone (**1b**), in particular, has an important place in the clinical management of leukemias and lymphomas as well as in combination therapy of advanced breast and ovarian cancers. Although the toxic side effects associated with **1b** appear to be less pronounced than in the case of doxorubicin, myelotoxicity and cardiotoxicity are still of clinical concern, especially in patients with prior treatment with doxorubicin [7,8].

The design of new antitumor drugs is seriously hampered by lack of any clearly defined cellular target and the complexity of the mechanistic sequence of events which lead to cellular destruction. DNA intercalation appears to be a necessary event prior to the cell killing events for the anthracene-9,10-diones **1a** and **1b** [9,10].

Both **1a** and **1b** show cross-resistance to cell phenotypes developing resistance against doxorubicin. Such multidrug resistance (MDR) involves a number of anticancer agents and contributes to therapeutical failure in the treatment of solid tumors with these agents [11-13]. Therefore, the search for novel anthracene-9,10-dione antitumor agents with higher therapeutic indices than **1b** is important. In addition, new drugs which are effective in the inhibition or delaying the growth of solid tumors which are more refractory towards chemotherapeutic agents (such as lung, breast and colon cancers) and against MDR tumor phenotypes must be developed.

As part of a drug development program leading to the synthesis and antitumor evaluations of bioisosteres related to the antitumor anthracene-9,10-diones, we have recently reported the synthesis and antitumor evaluations of analogues related to 6,9-bis[(aminoalkyl)amino]benz[*g*]-isoquinoline-5,10-diones **2** (Figure 1) [14]. Congener **2** [where $R_1 = R_2 = H$ (BBR 2778)] was found to be a highly effective antitumor agent showing high promise for clinical studies.



1a, X = H
b, X = OH

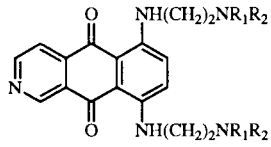


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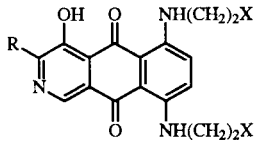
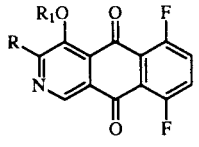
Figure 1

The synthesis of analogues related to **3** (Figure 2) which bear a hydroxy substituent at position 4 was accomplished in order to ascertain the importance of this substitution on antitumor activity.



3a, R = H, X = HNBOC
b, R = H, X = $N(CH_3)_2$
c, R = CH_3, X = $N(CH_3)_2$
d, R = H, X = NH_2



4a, R = R_1 = H
b, R = CH_3, R_1 = H
c, R = H, R_1 = $CH_2C_6H_5$
d, R = H, R_1 = $CH_2C_6H_4pOCH_3$

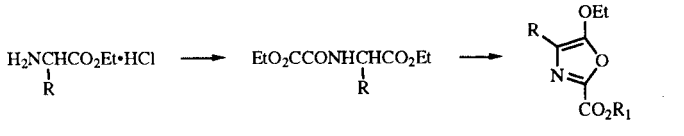
Figure 2

Synthesis.

The initial target molecule was the difluoride **4a** (Figure 2) which on treatment with the appropriate diamines would hopefully undergo formal S_NAr substitutions [5,14] to yield the desired analogues related to **3**. Two preparative routes to **4a** have been developed. Both are based on Kondrat'eva methodology for pyridine synthesis which utilizes oxazoles as synthons for azadienes [15-17].

The synthesis of the oxazole **7b**, which was utilized in both synthetic routes, is outlined in Scheme 1.

Scheme 1



5a, R = H
b, R = CH_3

6a, R = H
b, R = CH_3

7a, R = H, R_1 = Et
b, R = R_1 = H
c, R = CH_3, R_1 = Et
d, R = CH_3, R_1 = H

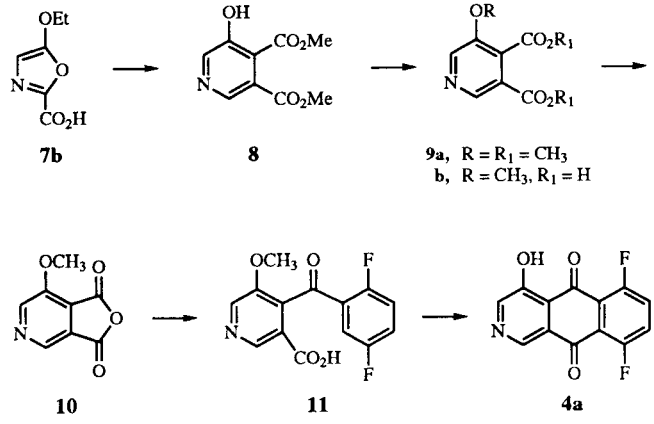
Oxazole **7a** (90%) was prepared by cyclodehydration of ethyl *N*-ethoxalylglycinate (**6a**) with phosphorus pentoxide in acetonitrile. Glycinate **6a** was readily available by treatment of ethyl glycinate hydrochloride (**5a**) with ethyl oxalyl chloride in refluxing benzene [18] or with diethyl oxalate in the presence of triethylamine [19]. Treatment of **7a** with aqueous sodium hydroxide led to the acid **7b** [17].

Oxazole **7c** (*vide infra*) was obtained by a similar pathway by cyclodehydration of **6b** with phosphorus oxychloride. Alaninate **6b** was prepared by treatment of ethyl alaninate hydrochloride (**5b**) with diethyl oxalate [19]. Treatment of ester **7c** with aqueous sodium hydroxide led to acid **7d.**

Pathway 1:

This route, based on a directed metalation strategy, is outlined in Scheme 2.

Scheme 2



7b **8** **9a**, R = R_1 = CH_3
b, R = CH_3, R_1 = H

10 **11** **4a**

Thermolysis of **7b** (an initial decarboxylation occurs to form the corresponding ethoxy oxazole) with dimethyl maleate at 95° led to **8** (76%). The addition of an ethereal solution of diazomethane to a methanol solution of **8** gave the methyl ether **9a** (80%). The choice of solvent was critical in order to circumvent the alkylation of the nitrogen in the pyridine ring (ether as solvent led to a 60:40 mixture of the *N*-methyl: *O*-methyl product mixture) [20-23]. The diester **9a** was hydrolyzed to the diacid **9b** (92%) which was converted into the anhydride **10** (77%) by refluxing in acetic anhydride [23].

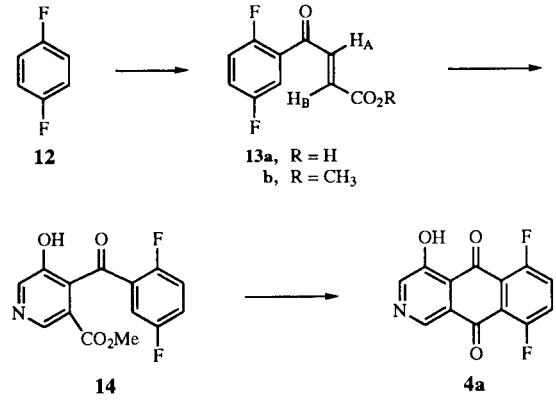
Treatment of the anhydride **10** with 2-lithio-1,4-difluorobenzene in THF at -78° provided 4-(2,5-difluorobenzoyl)-5-methoxynicotinic acid (**11**, 80% yield) in high regiospecificity. Prior investigators have reported regioselective attacks by lithium nucleophiles at the C=O at position 4 in pyridine-3,4-dicarboxylic anhydride [24-25].

Upon heating keto acid **11** with fuming sulfuric acid (27-33% SO_3) for 40 minutes at 135°, **4a** (85-90%) was obtained. Longer reaction times or higher reaction temperatures tended to lower the yields significantly.

Pathway 2:

An alternative and shorter synthesis of **4a** is described in Scheme 3.

Scheme 3



12 **13a**, R = H
b, R = CH_3

14 **4a**

Friedel-Crafts acylation of 1,4-difluorobenzene (**12**) with maleic anhydride and aluminum chloride led to *E*-acid **13a** (85%) [26-27]. In the ^{1}H nmr spectrum, protons H_A and H_B appeared at δ 6.98 (d, J_{AB} = 15.5 Hz) and δ 7.84 (dd, J_{BF} = 3.0 Hz, J_{AB} = 15.5 Hz), respectively. Treatment of **13a** with refluxing methanol in the presence of a small amount of concentrated sulfuric acid led predominantly to *E*-ester **13b** [28-29]. In addition to the absorption peak at δ 3.84 (s, OCH_3 for **13b**), four much less intense singlets appeared in the crude ^{1}H nmr spectrum. These singlets can be attributed to Michael-type additions of methanol to the double bond of **13a** (or **13b**) or to the carbonyl group followed by lactonization [29]. Pure methyl ester **13b** could be obtained from the mixture by chromatography, or more easily, by crystallization at low temperature from hexane (57%). The best preparative route to this ester (86% yield) involved treatment of acid **13a** with methyl chloroformate in the presence of triethylamine followed by addition of methanol.

The *E*-ester **13b** was heated with oxazole **7b** to yield, after crystallization from ethyl acetate, methyl 5-hydroxy-4-(2,5-difluorobenzoyl)nicotinate (**14**, 50%). Although two regioisomers are possible from this cycloaddition process, electronic considerations appear to energetically favor the transition state leading to **14** [30]. The structure was firmly extablished by X-ray crystallographic analysis [31] and the ORTEP diagram is shown in Figure 3. Pertinent crystallographic data are tabulated in Tables 1, 2, 3, 4 and 5.

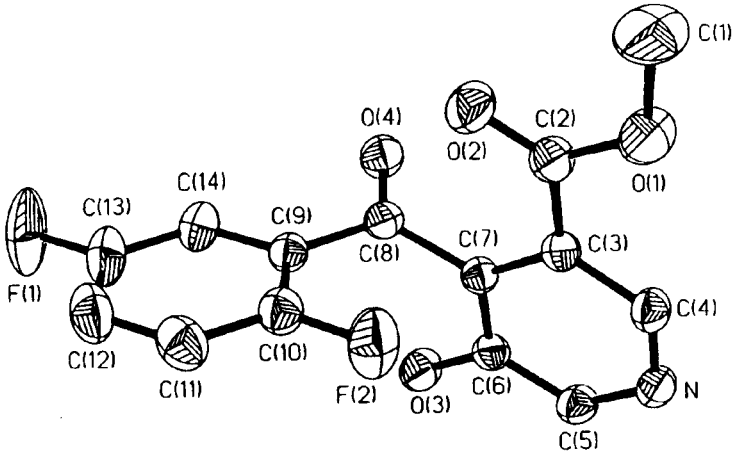


Figure 3. X-ray Crystallographic Structure of Regioisomer **14**.

Ester **14** could be converted into **4a** (81%) by heating in fuming sulfuric acid (27-33% sulfur trioxide) at 127° for 1 hour.

However, at this stage attempts to prepare the 4-hydroxy-6,9-bis[(aminoalkyl)amino-substituted benz[*g*]-isoquinoline-5,10-diones **3** from **4a** were unsuccessful.

Table 1

Single-Crystal X-ray Crystallographic Analysis

A. Crystal Parameters

formula	$C_{14}H_{11}F_2NO_5$(311.2)
crystallization medium	ethyl acetate
crystal size, mm	0.35 x 0.30 x 0.40
cell dimensions	a = 9.912(2) Å
	b = 11.263(2) Å
	c = 13.646(3) Å
	α = 90.00°
	β = 110.41(1)°
	Y = 90.00°
	V = 1427.8(5) Å^3
space group	$P2_1/n$
molecules/unit cell	4
density calcd, g/cm^3	1.448
linear absorption factor, cm^{-1}	1.27

B. Refinement Parameters

number of reflections	2515
nonzero relfections (I >3.0σ)	2058
R-index [a]	0.036
GOF [b]	1.15
secondary extinction factor	1.6(5) x 10^{-3}

[a] R-index = $\Sigma \| Fo \| - \| Fc \| / \Sigma \| Fo \|$. [b] GOF = $[\Sigma w (Fo^2-Fc^2)^2/(m-s)]^{1/2}$ where $w = [\sigma^2(F) + |g|F^2]^{-1}$ g = 0.002

Table 2

Bond Lengths (Å)

F(1)-C(13)	1.350 (3)	F(2)-C(10)	1.346 (2)
O(1)-C(1)	1.454 (3)	O(1)-C(2)	1.318 (2)
O(2)-C(2)	1.196 (2)	O(3)-C(6)	1.342 (2)
O(4)-C(8)	1.213 (2)	N-C(4)	1.332 (2)
N-C(5)	1.332 (2)	C(2)-C(3)	1.490 (2)
C(3)-C(4)	1.383 (2)	C(3)-C(7)	1.393 (2)
C(5)-C(6)	1.392 (2)	C(6)-C(7)	1.394 (2)
C(7)-C(8)	1.509 (2)	C(8)-C(9)	1.485 (3)
C(9)-C(10)	1.383 (3)	C(9)-C(14)	1.396 (2)
C(10)-C(11)	1.377 (3)	C(11)-C(12)	1.371 (3)
C(12)-C(13)	1.372 (4)	C(13)-C(14)	1.373 (4)

Table 3

Bond Angles (°)

C(1)-O(1)-C(2)	116.6 (2)	C(4)-N-C(5)	118.4 (1)
O(1)-C(2)-O(2)	124.9 (2)	O(1)-C(2)-C(3)	111.8 (1)
O(2)-C(2)-C(3)	123.4 (2)	C(2)-C(3)-C(4)	121.7 (1)
C(2)-C(3)-C(7)	119.2 (1)	C(4)-C(3)-C(7)	119.1 (1)
N-C(4)-C(3)	122. 7 (2)	N-C(5)-C(6)	123.1 (1)
O(3)-C(6)-C(5)	123.5 (1)	O(3)-C(6)-C(7)	118.2 (1)
C(5)-C(6)-C(7)	118.3 (1)	C(3)-C(7)-C(6)	118.3 (1)
C(3)-C(7)-C(8)	124.6 (1)	C(6)-C(7)-C(8)	117.0 (1)
O(4)-C(8)-C(7)	119.1 (2)	O(4)-C(8)-C(9)	121.0 (1)
C(7)-C(8)-C(9)	119.5 (2)	C(8)-C(9)-C(10)	125.3 (2)
C(8)-C(9)-C(14)	117.5 (2)	C(10)-C(9)-C(14)	117.3 (2)
F(2)-C(10)-C(9)	119.7 (2)	F(2)-C(10)-C(11)	117.2 (2)
C(9)-C(10)-C(11)	123.0 (2)	C(10)-C(11)-C(12)	119.3 (2)
C(11)-C(12)-C(13)	118.2 (2)	F(1)-C(13)-C(12)	118.7 (2)
F(1)-C(13)-C(14)	118.0 (3)	C(12)-C(13)-C(14)	123.4 (2)
C(9)-C(14)-C(13)	118.8 (2)		

Table 4

Anisotropic Displacement Coefficients ($Å^2 \times 10^3$)

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
F(1)	156(2)	33(1)	196(2)	-16(1)	98(2)	-4(1)
F(2)	114(1)	48(1)	102(1)	-4(1)	79(1)	-12(1)
O(1)	62(1)	51(1)	78(1)	21(1)	-15(1)	-7(1)
O(2)	48(1)	51(1)	63(1)	-6(1)	3(1)	-3(1)
O(3)	51(1)	39(1)	53(1)	11(1)	4(1)	-2(1)
O(4)	63(1)	44(1)	46(1)	-1(1)	26(1)	-6(1)
O(5)	61(1)	50(1)	51(1)	-18(1)	8(1)	-3(1)
N	46(1)	35(1)	45(1)	-3(1)	10(1)	-5(1)
C(1)	70(2)	85(2)	89(2)	36(2)	-23(2)	-10(2)
C(2)	42(1)	40(1)	44(1)	4(1)	12(1)	0(1)
C(3)	38(1)	33(1)	39(1)	2(1)	13(1)	1(1)
C(4)	44(1)	32(1)	45(1)	3(1)	13(1)	-1(1)
C(5)	40(1)	43(1)	41(1)	0(1)	7(1)	-2(1)
C(6)	41(1)	34(1)	36(1)	5(1)	12(1)	2(1)
C(7)	38(1)	33(1)	34(1)	-1(1)	14(1)	0(1)
C(8)	36(1)	34(1)	38(1)	4(1)	11(1)	-1(1)
C(9)	35(1)	33(1)	40(1)	0(1)	9(1)	1(1)
C(10)	51(1)	41(1)	48(1)	0(1)	19(1)	-1(1)
C(11)	55(1)	64(1)	60(1)	-4(1)	28(1)	8(1)
C(12)	58(1)	58(1)	77(1)	-12(1)	24(1)	15(1)
C(13)	67(1)	36(1)	92(2)	-7(1)	27(1)	5(1)
C(14)	52(1)	36(1)	67(1)	-3(1)	22(1)	-5(1)

The anisotropic displacement exponent takes the form:

$-2p^2 (h^2 a^{*2} U_{11} + ... + 2hka^*b^* U_{12})$

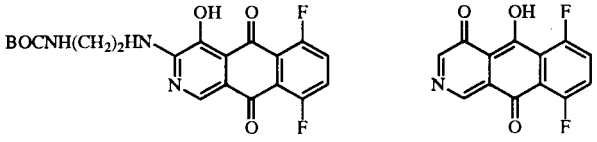
Table 5

Atomic Coordinates ($x10^4$) and Equivalent Isotropic Displacement Coefficients ($Å^2 \times 10^3$)

	x	y	z	U (eq)
F(1)	2073(2)	2627(1)	10473(2)	119(1)
F(2)	1873(2)	7280(1)	11330(1)	77(1)
O(1)	1238(2)	9935(1)	9121(1)	74(1)
O(2)	1153(1)	7966(1)	8937(1)	58(1)
O(3)	6039(2)	6784(1)	11499(1)	51(1)
O(4)	3740(1)	6294(1)	9213(1)	49(1)
O(5)	6258(2)	2054(2)	2347(1)	57(1)
N	5109(2)	9906(1)	11449(1)	43(1)
C(1)	-180(4)	10040(3)	8318(3)	94(1)
C(2)	1754(2)	8851(2)	9349(1)	43(1)
C(3)	3206(2)	8854(1)	10181(1)	37(1)
C(4)	3822(2)	9886(2)	10692(1)	41(1)
C(5)	5835(2)	8892(2)	11714(1)	43(1)
C(6)	5312(2)	7810(1)	11241(1)	38(1)
C(7)	3954(2)	7787(1)	10463(1)	35(1)
C(8)	3423(2)	6604(1)	9957(1)	37(1)
C(9)	2666(2)	5785(1)	10445(1)	37(1)
C(10)	1935(2)	6125(2)	11098(1)	46(1)
C(11)	1230(2)	5331(2)	11518(2)	58(1)
C(12)	1276(2)	4144(2)	11312(2)	64(1)
C(13)	2015(3)	3795(2)	10678(2)	65(1)
C(14)	2693(2)	4575(2)	10229(2)	51(1)
Hc(5)	6780(20)	8904(16)	12269(15)	42(5)
Hc(4)	3383(19)	10639(18)	10523(13)	40(4)
Ho(3)	6976(33)	7005(25)	11941(22)	88(8)
Hc(14)	3207(24)	4329(22)	9809(17)	66(6)
Hc(11)	694(25)	5628(23)	11944(19)	77(7)
Hc(12)	787(29)	3582(25)	11585(21)	85(8)

* Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor.

Treatment of **4a** with *N,N*-dimethylethylenediamine in dimethyl sulfoxide led to numerous products from which **3b** could not be isolated. On the other hand, dione **4a** reacted with *N*-(*t*-butoxycarbonyl)ethylenediamine in dimethyl sulfoxide to yield **15** (Figure 4) which was readily characterized by ^1H nmr and mass spectral analysis.



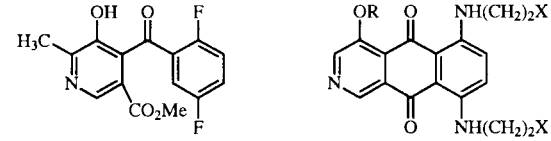
15 **16**

Figure 4

The formation of **15** probably proceeds *via* an initial Michael-type addition of the amine electron pair of the primary amino functionality to C-3 of the tautomer **16**, or the anionic form, (Figure 4) followed by tautomerization to the aromatic azaanthracene skeleton and spontaneous air oxidation during the workup.

To further probe into the problem encountered in the diplacements reactions on **4a**, we synthesized the methyl analogue **4b**. Thermolysis of a mixture of **7d** and **13b** led to ester **17** (40%) (Figure 5). This structure is based on the anticipated similar regiochemistry of this cycloaddition to that found in the formation of **14.** Upon heating ester **17** in fuming sulfuric acid (30% sulfur trioxide), **4b** (66%) was obtained.

Treatment of **4b** with *N,N*-dimethylethylene diamine led to the bis-substitution product **3c**, clearly indicating the importance of the hydrogen atom at C-3 in the pyridine ring for substitution in **4a** at this position.



17

18a, R = $CH_2C_6H_5$, X = NHBOC
b, R = $CH_2C_6H_5$, X = $N(CH_3)_2$
c, R = $CH_2C_6H_4pOCH_3$, X = $N(CH_3)_2$
d, R = $CH_2C_6H_4pOCH_3$, X = NHBOC

Figure 5

A protective group for the phenolic moiety was then sought which would allow facile placement and removal after the displacements were accomplished. Attempts to prepare the tetrahydropyran or silyl ether derivatives from **4a** were unsuccessful. Acylation with either acetic or pivalic anhydride did provide the corresponding derivatives; however, both protecting groups were removed by

the diamines under conditons used to effect the displacements.

Attempts to benzylate the phenol moiety in **4a** using benzyl bromide were unsuccessful since competitive benzylation occurred at the ring nitrogen. Treatment of the dione **4a** with ethereal phenyl diazomethane [32] led to the benzyl ether **4c** (61%). Treatment of **4c** with *N*-(*t*-butoxycarbonyl)ethylene diamine in dimethyl sulfoxide or with *N,N*-dimethylethylene diamine in pyridine gave the corresponding 6,9-bis-substituted analogues **18a** (61%) and **18b** (87%), respectively (Figure 5).

Debenzylations of **18a** or **18b** using palladium hydroxide on carbon (Pearlman's catalyst) and hydrogen gas, followed by air oxidation led to the hydroxy analogues **3a** (87%) and **3b** (61%), respectively. During the hydrogenolysis, the leuco analogues related to **3a** and **3b** were formed which necessitated an oxidation step. The conversion of the protected bis-BOC diamine **3a** into the dione **3d** (as its hydrochloride salt) was accomplished in near quantitative yields by treating **3a** with anhydrous hydrogen chloride gas in dichloromethane.

The hazardous nature of phenyl diazomethane prompted us to explore the *p*-methoxybenzyl group as the protective group for the phenolic moiety, which after displacements of the fluorides would be readily removed under acidic conditions [33]. Following a procedure similar to that used for the benzylation of pyridinols [34-36], treatment of **4a** with *O-p*-methoxybenzyl-*N,N'*-disopropylisourea (prepared *via* treatment of diisopropylcarbodiimide with *p*-methoxybenzyl alcohol [37]) led to **4d** (44%). Treatment of **4d** with *N,N*-dimethylethylenediamine in pyridine or with *N*-(*t*-butoxycarbonyl)ethylenediamine in *N*-methylpyrrolidinone led to **18c** (42%) and **18d** (58%), resepctively. The removal of the *p*-methoxybenzyl group was accomplished by treatment of **18c** with methanesulfonic acid in dichloromethane to yield **3b** (51%). Treatment of **18d** with trifluoroacetic acid removed both protective groups and led to **3d** as the dimaleate salt upon treatment of the crude deprotected product with maleic acid.

The antitumorigenic properties of the compounds will be described in a subsequent publication.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Proton nmr were run on a Bruker WP-270SY, WM-250 or ARX-500 pulsed Fourier transform spectrometer. Precoated silica gel plates (Eastman Chromagram sheets) with fluorescent indicator were used for thin layer chromatography. Baker analyzed 80-200 mesh silica gel was used for column chromatography and 230-400 mesh for flash chromatography. Microanalyses were performed by Robertson Laboratory, Madison, NJ or by Redox s.n.c., Cologno Monzese, Milan, Italy.

4-Hydroxy-6,9-bis[[2-[*N*-(*t*-butoxycarbonyl)amino]ethyl]-amino]benz[*g*]isoquinoline-5,10-dione (**3a**).

A mixture of **18a** (135 mg, 0.213 mmole) and Pearlman's catalyst (29 mg) in methanol:acetic acid (4:1; 5.8 ml) was stirred under a positive pressure of hydrogen for 15 minutes. The orange mixture was reoxidized by a blanket of oxygen for 13 hours. The solvents were removed under a nitrogen stream and the resultant mixture was placed under vacuum for 2 hours. The blue mixture was taken up in chloroform and filtered through celite. Purification by column chromatography (silica gel: 2.0 x 15 cm) by gradient elution with chloroform:methanol (99:1, 98:2, 96:4, 90:10) led to a major blue band. The removal of the eluent by rotary evaporation gave **3a**, 112 mg (97%), mp 208-211°; ^{1}H nmr (deuteriochloroform): δ 12.55 (s, 1H), 10.98 (br, 1H), 10.45 (br, 1H), 8.56 (s, 1H), 8.23 (s, 1H), 7.00 (d, 1H), 6.92 (d, 1H), 5.93 (br, 1H), 5.92 (br, 1H), 3.44 (m, 8H), 1.49 (s, 18H).

Anal. Calcd. for $C_{27}H_{35}N_5O_7$: C, 59.88; H, 6.51; N, 12.93. Found: C, 59.48; H, 6.40; N, 13.20.

4-Hydroxy-6,9-bis[[2-(dimethylamino)ethyl]amino]benz[*g*]isoquinoline-5,10-dione (**3b**).

A. From **18b**:

A mixture of **18b** (50 mg, 0.102 mmole) and Pearlman's catalyst (12 mg) in methanol:acetic acid (4:1, 1.0 ml) was stirred under a positive pressure of hydrogen for 0.75 hour. The brick red leuco mixture was reoxidized with a gentle stream of oxygen for 10 hours after addition of methanol (4 ml). The solvents were removed under reduced pressure and the blue mixture was taken up in chloroform and filtered through a celite bed. Purification by column chromatography (silica gel, 2.0 x 4 cm) with gradient eution by chloroform:methanol [90:10, 70:30 (150 ml)] eluted a major blue band. The removal of the eluent by rotary evaporation gave a blue solid, 25 mg (62%), mp 217-218°; ^{1}H nmr (deuteriochloroform): δ 12.74 (s, 1H), 11.12 (br, 1H), 10.71 (br, 1H), 9.05, (s, 1H), 8.58 (s, 1H), 7.28 (m, 2H), 3.51 (m, 4H), 2.69 (m, 4H), 2.33, (s, 6H), 2.32 (s, 6H).

Anal. Calcd. for $C_{21}H_{27}N_5O_3 \cdot H_2O$: C, 60.70; H, 7.04; N, 16.86. Found: C, 60.35; H, 7.00; N, 16.76.

B. From **18c**:

Methanesulfonic acid (20 drops) was added to the blue solution of **18c** (28 mg, 0.05 mmole) in dichloromethane (1 ml) upon which an immediate purple coloration developed. The mixture was stirred at room temperature for 1 hour and quenched by dropwise addition of a saturated sodium bicarbonate solution (1 ml) whereupon a blue coloration developed. The dichloromethane layer was separated and the aqueous phase extracted with dichloromethane (5 ml). The extracts were washed with water (2 x 2 ml), dried over sodium sulfate and concentrated to dryness to yield crude product (20 mg). This was purified by column chromatography over silica gel (1.75 x 12 cm) with methanol:dichloromethane mixtures progressively containing 4% (200 ml), 8% (100 ml), 12% (100 ml), 25% (100 ml), 30% (300 ml), and 35% (100 ml) of methanol. The major blue product band began eluting with 25% methanol: 75% dichloromethane. The combined fractions were concentrated to yield a blue solid, 11 mg (51%).

3-Methyl-4-hydroxy-6,9-bis[[2-(dimethylamino)ethyl]amino]-benz[*g*]isoquinoline-5,10-dione (**3c**).

A solution of **4b** (40 mg, 0.14 mmole) and *N,N*-dimethylethylenediamine (246 mg, 2.8 mmoles) in pyridine (1 ml) was stirred at room temperature for 18 hours. The pyridine and excess amine were removed by a gentle nitrogen stream and the blue residue was placed under vacuum for 4 hours. The blue residue was purified by column chromatography (silica gel, 1.75 x 12 cm, 225 mesh) using gradient elution mixtures of methanol: dichloromethane progressively containing 10%, 15%, 20%, 25%, 30%, 40% and 50% of methanol. Removal of solvents by rotary evaporation yielded a blue solid, 14 mg (25%), mp 147-148°; ^{1}H nmr (deuteriochloroform): δ 13.21 (s, 1H), 10.99 (m, 1H), 10.63 (m, 1H), 8.96 (s,1H), 7.31 (d, J_{HH} = 9.8 Hz, 1H), 7.22 (d, J_{HH} = 9.8 Hz, 1H), 3.52 (t, J_{HH} = 5.8 Hz, 4H), 2.66 (m, 4H), 2.63 (s, 3H), 2.36 (s, 6H), 2.34 (s, 6H).

Anal. Calcd. for $C_{22}H_{29}N_5O_3 \cdot 2H_2O$: C, 59.04; H, 7.43; N, 15.64. Found: C, 58.80; H, 7.40; N, 15.30.

4-Hydroxy-6,9-bis[(2-aminoethyl)amino]benz[*g*]isoquinoline-5,10-dione (**3d**).

Route 1: (Hydrochloride Salt from **3a**).

A stream of dry hydrogen chloride gas was placed over a stirred solution of **3a** (26 mg, 0.049 mmole) in chloroform. A positive pressure of the gas was maintained over the reaction for 20 minutes. The blue solid, 20 mg (99%) was collected by filtration, mp 212-214°; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 12.91 (s, 1H), 11.07 (br, 1H), 10.64 (br, 1H), 8.86 (s, 1H), 8.56 (s, 1H), 7.71 (m, 2H), 3.83 (m, 4H), 3.05 (m, 4H).

Anal. Calcd. for $C_{17}H_{19}N_5O_3 \cdot 3HCl \cdot 2H_2O$: C, 41.95; H, 5.38; N, 14.39. Found: C, 42.13; H, 5.20; N, 14.05.

Route 2: (Dimaleate Salt from **18d**).

Under a nitrogen atmosphere, trifluoroacetic acid (2.36 ml) was added to a stirred solution of **18d** (1.0 g, 1.51 mmoles) in dichloromethane (20 ml) at room temperature. After stirring overnight, the solvent was removed by rotary evporation. The residue was dissolved in absolute ethanol (2.05 ml) and maleic acid (0.41 g, 3.47 mmoles) was added to the dark blue stirred solution. After 1 hour at room temperature and 3 hours at 0°, the blue precipitate was collected by filtration, washed with ethanol and dried under vacuum, 850 mg (98%), mp 171-173°; ^{1}H nmr (deuterium oxide): δ 8.50 (s, 1H), 8.30 (s, 1H), 7.2-7.3 (m, 2H), 6.20 (s, 4H), 3.80 (t, 2H), 3.85 (t, 2H), 3.40 (t, 4H),

Anal. Calcd. for $C_{17}H_{19}N_5O_3 \cdot 2C_4H_4O_4 \cdot 2H_2O$: C, 49.26: H, 5.12: N, 11.49. Found: C, 49.16; H, 5.00; N, 11.42.

4-Hydroxy-6,9-difluorobenz[*g*]isoquinoline-5,10-dione (**4a**).

Method A:

The keto acid **11** (48 mg, 0.164 mmole) was heated with fuming sulfuric acid (27-33% SO_3, 0.2 ml) for 45 minutes at 135°. The golden-brown solution was quenched over ice water (5 ml) and extracted with dichloromethane (6 x 10 ml). The extracts were dried with sodium sulfate and the dichloromethane was removed by rotary evaporation. The yellow solid weighed 40 mg (94%), mp 221-222°; ^{1}H nmr (deuteriochloroform): δ 11.61 (s, 1H), 9.03 (s, 1H), 8.84 (s, 1H), 7.59 (m, 2H).

Anal. Calcd. for $C_{13}H_5F_2NO_3$: C, 59.78; H, 1.93; N, 5.36. Found: C, 59.50; H, 1.78; N, 5.08.

Method B:

The keto ester **14** (500 mg, 1.71 mmoles) and fuming sulfuric acid (90 drops, 27-33% sulfur trioxide) were stirred in an oil bath at 127° for 1 hour. The mixture was quenched onto ice water and extracted with dichloromethane (3 x 30 ml). The extract was dried over sodium sulfate and the solvent removed by rotary evaporation to yield an orange-yellow solid, 407 mg (91%), mp 219-221°.

3-Methyl-4-hydroxy-6,9-difluorobenz[*g*]isoquinoline-5,10-dione (**4b**).

Ester **17** (50 mg, 0.16 mmole) was placed in fuming sulfuric acid (30% sulfur trioxide, 0.25 ml) and heated for 40 minutes in an oil bath held at 130-135°. The dark red solution was quenched over ice (5 g) and extracted with dichloromethane (20 ml). The aqueous layer was carefully neutralized with solid sodium bicarbonate and extracted with dichloromethane (2 x 15 ml). The combined extracts were dried over magnesium sulfate and the solvent was removed by rotary evaporation to yield **4b**, 30 mg (66%) as a yellow solid, mp 158-159°; ^{1}H nmr (deuteriochloroform): δ 12.02 (s, 1H), 8.90 (s, 1H), 7.54 (m, 2H), 2.70 (s, 3H).

Anal. Calcd. for $C_{14}H_7F_2NO_3$: C, 61.10; H, 2.56; N, 5.09. Found: C, 61.10; H, 2.46; N, 4.87.

4-Benzyloxy-6,9-difluorobenz[*g*]isoquinoline-5,10-dione (**4c**).

An ethereal solution of phenyldiazomethane (15 ml, about 0.8 *M*) was added to **4a** (107 mg, 0.41 mmole) in methanol (10 ml) and tetrahydrofuran (10 ml). The mixture was stirred overnight (12 hours) and the solvents were removed from the resultant reddish-orange solution by rotary evaporation. The red tacky material was washed with hexane (20 ml) and ether (15 ml) and the brownish-yellow solid **4c**, 56 mg (39%) was collected by filtration, mp 210-212°; ^{1}H nmr (deuteriochloroform): δ 9.09 (s, 1H), 8.81 (s, 1H), 7.56 (m, 2H), 7.42 (m, 5H), 5.44 (s, 2H).

Anal. Calcd. for $C_{20}H_{11}F_2NO_3$: C, 68.38; H, 3.16; N, 3.99. Found: C, 67.98; H, 3.26; N, 3.59.

4-*p*-Methoxybenzyloxy-6,9-difluorobenz[*g*]isoquinoline-5,10-dione (**4d**).

A dark brown solution of dione **4a** (1.83 g, 7 mmoles) and *O*-*p*-methoxybenzyl-*N,N'*-diisopropylisourea (4.11 g, 15.5 mmoles) in dichloromethane (37 ml) was stirred for 16 hours at room temperature under a nitrogen blanket. The precipitated solid was removed by filtration and the filtrate washed with demineralized water (50 ml), dried over sodium sulfate and concentrated to dryness. The deep brown oily residue was chromatographed on a silica gel column (70-230 mesh, 3 x 20 cm) by using hexane:ethyl acetate mixtures from 5:1 to 2:1 (v:v). The product fractions were combined and the solvents removed under vacuum. The residue was suspended in a mixture of hexane: ethyl acetate 5:1 (20 ml) and stirred at room temperature for 1 hour. A yellow-brown solid was collected by filtration and dried to yield the protected derivative **4d**, 1.18 g (44%), mp 161-163°; ^{1}H nmr (deuteriochloroform): δ 9.10 (s, 1H), 8.82 (s, 1H), 7.46 (m, 2H), 7.35 (m, 2H), 6.95 (d, J_{HH} = 8.5 Hz, 2H), 5.38 (s, 2H), 3.84 (s, 3H).

Anal. Calcd. for $C_{21}H_{13}F_2NO_4$: C, 66.14; H, 3.44; N, 3.67. Found: C, 66.05; H, 3.45; N, 3.65.

Ethyl *N*-Ethoxalylglycinate (**6a**).

Procedure 1:

A stirred mixture of **5a** (100 g, 0.716 mole), diethyl oxalate (209 g, 1.43 moles) and triethylamine (100 ml, 0.716 mole) in ethanol (300 ml) was warmed to 50°. After 20 minutes all compo-

nents had dissolved and after 5 hours the solvent was removed under vacuum. The residual mass was partitioned between demineralized water (2 l) and chloroform (2 l) and the aqueous layer was extracted with chloroform (2 x 500 ml). After the removal of the solvent, the residue was distilled to yield **6a**, 125 g (86%), bp 144° (0.3 mm Hg), lit bp 188° (18 mm Hg) [18]; ^{1}H nmr (deuteriochloroform): δ 7.58 (m, 1H), 4.38 (q, J_{HH} = 7.1 Hz, 2H), 4.25 (q, J_{HH} = 7.1 Hz, 2H), 4.12 (d, J_{HH} = 5.5 Hz, 2H), 1.40 (t, J_{HH} = 7.1 Hz, 3H), 1.31 (t, J_{HH} = 7.1 Hz, 3H).

Procedure 2:

Compound **6a** (97% yield) was obtained from **5a** and ethyl oxalyl chloride following the literature procedure [18].

Ethyl *N*-Ethoxalylalaninate (**6b**).

A stirred solution of **5b** (5.0 g, 0.032 mole), ethanol (30 ml), triethylamine (3.3 g, 0.033 mole) and diethyl oxalate (9.5 g, 0.065 mole) was refluxed overnight under a nitrogen atmosphere. The cooled mixture was concentrated and the residue was partitioned between chloroform (30 ml) and water (20 ml). The organic layer was washed with water (15 ml), dried over magnesium sulfate and the solvent was removed by rotary evaporation to leave a pale yellow oil which was purified by vacuum distillation to yield a colorless liquid, 5.1 g (71%), bp 106-108° (0.5 mm Hg) lit bp 127-129° (2 mm Hg) [19]; ^{1}H nmr (deuteriochloroform): δ 7.59 (m, 1H), 4.60 (m, 1H), 4.38 (q, J_{HH} = 7.0 Hz, 2H), 4.20 (q, J_{HH} = 7.0 Hz, 2H), 1.48 (d, J_{HH} = 7.1 Hz, 3H), 1.38 (t, J_{HH} = 7.0 Hz, 3H), 1.30 (t, J_{HH} = 7.0 Hz, 3H).

5-Ethoxy-2-ethoxycarbonyloxazole (**7a**).

Under a nitrogen atmosphere a solution of **6a** (125 g, 0.615 mole) in dry acetonitrile (125 ml) was added dropwise to a vigorously stirred suspension of phosphorus pentoxide (436 g, 3.07 moles) in acetonitrile (1250 ml) previously warmed to 35-40°. The mixture was heated to 65-70° for 1 hour. The white suspension was cooled to 5°, quenched into a mixture of ice and brine (15 l) and the product extracted with ethyl acetate (3 x 2 l). The combined extracts were washed with brine (2 l), cold 5% sodium bicarbonate (2 l) and dried over sodium sulfate. After removal of the solvents, the residual oil was distilled under vacuum to yield a viscous oil which crystallized spontaneously, 103.5 g (91%), bp 113° (0.30 mm Hg); ^{1}H nmr (deuteriochloroform): δ 6.32 (s, 1H), 4.41 (q, J_{HH} = 7.0 Hz, 2H), 4.24 (q, J_{HH} = 7.2 Hz, 2H), 1.44 (t , J_{HH} = 7.2 Hz, 3H), 1.39 (t, J_{HH} = 7.0 Hz, 3H).

5-Ethoxy-2-carboxyoxazole (**7b**).

Oxazole **7a** (2.0 g, 0.011 mole) was added portionwise to a solution of sodium hydroxide (1.08 g, 0.027 mole) in water (6.7 ml). The mixture was stirred at room temperature for 1 hour, cooled in an ice bath and acidified with concentrated hydrochloric acid to *p*H = 2. The precipitated white solid was collected by filtration, washed with water (2 x 3 ml) and dried in a desiccator over phosphorus pentoxide to yield **7b**, 1.5 g (90%), mp 101-102° dec, lit mp 97-98° dec [17]; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 6.60 (s, 1H), 4.23 (q, J_{HH} = 7.0 Hz, 2H), 1.34 (t, J_{HH} = 7.0 Hz, 3H).

5-Ethoxy-2-ethoxycarbonyl-4-methyloxazole (**7c**).

To a stirred solution of **6b** (4 g, 0.018 mole) and triethylamine (5.6 g, 0.055 mole), cooled to -10° under a nitrogen atmosphere in toluene (10 ml), a solution of phosphorus oxychloride (3.4 g, 0.018 mole) in toluene (10 ml) was added dropwise over a period of 1 hour. The mixture was heated to reflux overnight. The cooled reaction mixture was filtered and the residue was rinsed with toluene (2 x 15 ml), the filtrate was washed with water (2 x 15 ml) and dried over sodium sulfate. The solvent was removed by rotary evaporation to leave a brown viscous oil which was purified by vacuum distillation to yield **7c**, 3 g (82%) as a pale yellow liquid which solidified on cooling, bp 92-95° (0.5 mm Hg), lit 128° [19]; ^{1}H nmr (deuteriochloroform): δ 4.42 (q, J_{HH} = 7.1 Hz, 2H), 4.38 (q, J_{HH} = 7.1 Hz, 2H), 2.11 (s, 3H), 1.40 (t, J_{HH} = 7.1 Hz, 6H).

4-Methyl-5-ethoxy-2-carboxyoxazole (**7d**).

Ester **7c** (1 g, 0.005 mole) was added to a solution of sodium hydroxide (0.4 g, 0.01 mole) in water (2 ml). The mixture was stirred at room temperature for 1 hour, cooled in an ice bath and acidified with concentrated hydrochloric acid to *p*H = 2. The precipitated white solid was collected by filtration, washed with ice water (1 ml) and dried in a desiccator over phosphorus pentoxide to yield **7d**, 0.81 g (95%), mp 82-83°, lit mp 83-84° [19]; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 4.27 (q, J_{HH} = 7.1 Hz, 2H), 2.02 (s, 3H), 1.30 (t, J_{HH} = 7.1 Hz, 3H).

Dimethyl 3-Hydroxypyridine-4,5-dicarboxylate (**8**).

A stirred mixture of **7b** (7.09 g, 0.045 mole) and dimethyl maleate (12.99 g, 0.090 mole) was heated at 100° for 5 hours under a nitrogen atmosphere. The evolution of carbon dioxide gas commenced at 70°. The crystalline material which separated from the reaction mixture on standing overnight was collected by filtration and washed with cold acetone to yield **3a** as a pale yellow solid, 7.25 g (76%), mp 137-139°, lit mp 129-133° [38]; ^{1}H nmr (deuteriochloroform): δ 10.03 (br s, 1H), 8.57 (s, 1H), 8.31 (s, 1H), 3.98 (s, 3H), 3.92 (s, 3H).

Dimethyl 3-Methoxypyridine-4,5-dicarboxylate (**9a**).

An ethereal solution of diazomethane was added in small portions to a solution of **8** (2.55 g, 0.012 mole) in methanol (77 ml) until a permanent yellow coloration persisted. After about 2 hours the solvents were removed by rotary evaporation to yield a golden oil. Purification by column chromatography (silica gel: 2.25 x 18 cm) with ethyl acetate as the eluent (450 ml) yielded **9a** as a white solid, 2.20 g (80%), mp 76-77°, lit mp 78-80° [23]; ^{1}H nmr (deuteriochloroform): δ 8.86 (s, 1H), 8.52 (s, 1H), 3.97 (s, 3H), 3.96 (s, 3H), 3.92 (s, 3H).

3-Methoxypyridine-4,5-dicarboxylic Acid (**9b**).

The diester **9a** (1.71 g, 0.0076 mole) was added to a magnetically stirred solution of sodium hydroxide (1.28 g, 0.032 mole) in water (5.6 ml). The mixture was heated to 95° for 1 hour and then cooled in an ice bath. Concentrated hydrochloric acid was added dropwise to the mixture and the white solid which separated was collected by filtration and washed with ice water (2 ml). A second crop was obtained by further acidification of the filtrate and filtration which on combination with the first crop led to **9b**, 1.38 g (92%), mp 224-225°, lit mp 218 -220° [23]; ^{1}H nmr (dimethyl sulfoxide-d_6): δ 13.54 (br, 2H), 8.68 (s, 1H), 8.67 (s, 1H), 3.94 (s, 3H).

3-Methoxypyridine-4,5-dicarboxylic Anhydride (**10**).

A mixture of diacid **9b** (1.34 g, 0.0070 mole) and acetic anhydride (40 ml) was heated to reflux for 0.5 hour. The excess anhydride was removed by distillation under reduced pressure. Purification by vacuum sublimation (110°, 0.12 mm (Hg)) yielded a pale yellow solid, 0.938 g (77%), mp 158-159°, lit mp

160-161° [23]; ^{1}H nmr (deuteriochloroform): δ 8.91 (s, 1H), 8.83 (s, 1H), 4.23 (s, 3H).

5-Methoxy-4-(2,5-difluorobenzoyl)nicotinic Acid (**11**).

Sec-butyllithium (1.32 *M* in cyclohexane; 0.92 ml, 1.21 mmoles) was added dropwise *via* a syringe to a stirred solution of 1,4-difluorobenzene (136 mg, 1.20 mmoles) in THF (20 ml) at -77° which was kept under a nitrogen atmosphere. After stirring for about 20 minutes, the yellow mixture was transferred dropwise *via* a jacketed cannula into a stirred solution of **10** (196 mg, 1.09 mmoles) in THF (50 ml) at -78° under a nitrogen blanket. The amber solution was allowed to warm to room temperature and stirred for 19 hours. The THF was removed under a slow stream of nitrogen and the residual brown solid was taken up in water (2 ml) and stirred in an ice bath. The amber solution was acidified with concentrated hydrocloric acid and the resulting precipitate was collected by filtration and washed with ice water (5 ml) and ether (10 ml). The material was dissolved in hot chloroform and allowed to precipitate out slowly to yield a white solid, 251 mg (78%), mp 210-211°; ^{1}H nmr (acetone-d_6): δ 8.86 (s, 1H), 8.73 (s, 1H), 7.64 (m, 1H), 7.44 (m, 1H), 7.26 (m, 1H), 3.95 (s, 3H).

Anal. Calcd. for $C_{14}H_9F_2NO_4$: C, 57.34; H, 3.09; N, 4.78. Found: C, 57.05; H, 2.85; N, 4.66.

4-(2,5-Difluorophenyl)-4-oxo-2*E*-butenoic Acid (**13a**).

Maleic anhydride (6.0 g, 0.061 mole) was added over a 5-minute period to a stirred mixture of 1,4-difluorobenzene (40.0 ml, 44.0 g, 0.38 mole) and aluminum chloride (22.0 g, 0.16 mole). The mixture was placed in an oil bath which was rapidly heated to 100° during which time a vigorous evolution of hydrogen chloride occurred and the mixture developed an orange-reddish color. After 1 hour at this temperature, the excess 1,4-difluorobenzene (27 ml) was recovered by distillation and the dark residue was poured over ice and concentrated hydrochloric acid (18 ml). The yellow solid was collected by filtration and air dried (12 g). The crude material was taken up in boiling benzene (80 ml) and filtered to remove a small amount of insoluble material. The filtrate on cooling gave beautiful yellow crystals of **13a**, 11.0 g (85%) which were collected by filtration, mp 146-147°; ^{1}H nmr (deuteriochloroform): 10.21 (br s, 1H), 7.84 (dd, 1H, H-A, J_{HH} = 15.5 Hz, J_{HF} = 3.0 Hz), 7.54 (m, 1H), 7.33 (m, 1H), 7.19 (m, 1H), 6.98 (d, 1H, H-B, J_{HH} = 15.5 Hz).

Anal. Calcd. for $C_{10}H_6F_2O_3$: C, 56.61; H, 2.85. Found: C, 56.42; H, 2.56.

Methyl (2,5-Difluorophenyl)-4-oxo-2*E*-butenoate (**13b**).

Method A:

A solution of **13a** (2.2 g, 0.01 mole) in methanol (50 ml) containing concentrated sulfuric acid (12 drops) was refluxed for 2 hours. The mixture was cooled and the methanol was removed by rotary evaporation to leave a yellow oil. Ice water (50 ml) was added and a thick oily material formed. The ester was extracted with hexane (60 ml followed by 3 x 30 ml portions). The extracts were dried over sodium sulfate, partially concentrated to about 70 ml and the solution was placed in a freezer (-15°) for several days. The yellow solid was collected by filtration and washed with hexane to yield **13b**, 1.3 g (57%), mp 40-41°; ^{1}H nmr (deuteriochloroform): δ 7.74 (dd, H-A, J_{HH} = 15.5 Hz, J_{HF} = 3.1 Hz, 1H), 7.51 (m, 1H), 7.25 (m, 1H), 7.20 (m, 1H), 6.84 (dd, H-B, J_{HH} = 15.5 Hz, J_{HF} = 1 Hz, 1H), 3.84 (s, 3H).

Anal. Calcd. for $C_{11}H_8F_2O_3$: C, 58.41; H, 3.57. Found: C, 58.48; H, 3.23.

Method B:

Methyl chloroformate (25.8 ml, 0.33 mole) was added dropwise to a stirred solution of **13a** (50 g, 0.235 mole) and triethylamine (49.2 ml, 0.35 mole) in THF (500 ml) which was held at -5 to -10°. After 2 hours at -10°, methanol (500 ml) was added to the reaction mixture, the cooling bath was removed and the temperature allowed to reach 20°. After about 1 hour, the mixture was concentrated by rotary evaporation and the residue partitioned between water (500 ml) and ethyl acetate (3 x 200 ml). The extracts were washed with water, dried over sodium sulfate and concentrated. The residual brownish oil (56 g) was eluted from a silica gel column (70-230 mesh, 4 x 20 cm) with a petroleum ether: ethyl acetate mixture (8:2). After removal of the eluents, the orange oil which was obtained (47 g, 88%) spontaneously crystallized upon standing, mp 40-41° (a sample crystallized from methanol showed mp 48-49°).

Methyl 5-Hydroxy-4-(2,5-difluorobenzoyl)nicotinate (**14**).

A mixture of methyl ester **13b** (1.80 g, 0.0079 mole) and **7b** (1.30 g, 0.0082 mole) was heated under a nitrogen blanket to 90° in an oil bath. Carbon dioxide gas was evolved and the mixture was kept at this temperature for 4 hours. The reddish residue was cooled and placed on the rotary evaporator for 5 minutes. Chloroform was added and the crystalline product (1.5 g) was collected by filtration. This material was taken up in hot ethyl acetate and the mixture was filtered to remove a trace of insoluble material. Slow evaporation of the ethyl acetate led to crystals which were collected by filtration, 1.23 g (53%), mp 150-151°; ^{1}H nmr (deuteriochloroform): δ 11.42 (br s, 1H), 8.68 (s, 1H), 8.43 (s, 1H), 7.73 (m, 1H), 7.27 (m, 1H), 7.05 (m, 1H), 3.79 (s, 3H),

Anal. Calcd. for $C_{14}H_9F_2NO_4 \cdot H_2O$: C, 54.03; H, 3.56; N, 4.50. Found: C, 54.59; H, 3.45; N, 4.53.

3-[[2-(*N*-*tert*-Butoxycarbonyl)aminoethyl]amino]-4-hydroxy-6,9-difluorobenz[*g*]isoquinoline-5,10-dione (**15**).

A solution of (*N*-*tert*-butoxycarbonyl)ethylenediamine (78 mg, 0.049 mmole) in dimethyl sulfoxide (0.2 ml) was added to **4a** (12 mg, 0.046 mmole) upon which an immediate red coloration developed. The mixture was stirred at room temperature for 35 minutes under a nitrogen atmosphere. TLC analysis of a sample diluted with dichloromethane and applied to a silica gel plate eluted with dichloromethane:methanol:concentrated ammonium hydroxide (95:5:1) showed the disappearance of the starting material and the presence of a slower moving purple spot. The reaction mixture was quenched with saturated potassium phosphate monobasic (0.5 ml) and the purple precipitate was collected by filtration. The purple solid was taken up in dichloromethane (2 ml) and washed with water (3 x 1 ml), dried over magnesium sulfate and concentrated to dryness to yield **15**, 12 mg (68%), mp 192-193°. TLC analysis of the product using the eluent used above showed only 1 purple spot and the ^{1}H nmr spectrum indicated the presence of a trace amount of starting amine; ^{1}H nmr (deuteriochloroform): δ 8.60 (s, 1H), 7.52 (ddd, J_{HH} = 10.1 Hz, J_{HF} = 10.1 Hz, J_{HF} = 3.9 Hz, 1H), 7.43 (ddd, J_{HH} = 10.1 Hz, J_{HF} = 10.1 Hz, J_{HF} = 3.8 Hz, 1H), 3.74 (q, J_{HH} = 5.7 Hz, 2H), 3.46 (m, 2H), 1.17 (s, 9H); ms: (EI) M^+ 419.2; base peak [M - BOCNHCH]$^+$ 289.0.

Anal. Calcd. for $C_{20}H_{19}F_2N_3O_5$: C, 57.28; H, 4.57; N, 10.02. Found: C, 57.50; H, 4.85; N, 9.72.

Methyl 6-Methyl-4-(2,5-difluorobenzoyl)-5-hydroxynicotinate (**17**).

A mixture of **13b** (75 mg, 0.33 mmole) and **7d** (80 mg, 0.46 mmole) was heated to 60° for 0.5 hour. The cooled reaction mixture was purified by column chromatography (silica gel, 1.75 x 14 cm) using gradient elution commencing with hexane: ethyl acetate, 3:1, 200 ml, to 2:1 100 ml, 1:1 100 ml and ethyl acetate, which eluted the major yellow band. The solvents were removed by rotary evaporation to yield **17**, 40 mg (40%) as a pale yellow solid, mp 137-138°; ^{1}H nmr (deuteriochloroform): δ 8.59 (s, 1H), 7.40 (m, 1H), 7.24 (m, 1H), 7.05 (m, 1H), 3.60 (s, 3H), 2.63 (s, 3H).

Anal. Calcd. for $C_{15}H_{11}F_2NO_4$: C, 58.63; H, 3.61; N, 4.56. Found: C, 58.45; H, 3.93; N, 4.16.

4-Benzyloxy-6,9-Bis[[2-[*N*-(*t*-butoxycarbonyl)amino]ethyl]-amino]benz[*g*]isoquinoline-5,10-dione (**18a**).

A solution of **4c** (135 mg, 0.385 mmole) and *N*-(*t*-butoxycarbonyl)ethylenediamine (934 mg, 5.77 mmoles) in dimethyl sulfoxide (3.0 ml) was stirred at room temperature for 96 hours. The blue solution was quenched over ice (112 g) and the resulting precipitate was collected by filtration, washed with water (3 x 25 ml) and dried. Purification of the blue solid by column chromatography (silica gel, 1.25 x 14 cm) by gradient elution of chloroform:methanol [100:0, 98:2, 95:5 (200 ml)] led to a major blue band. The removal of the eluents by rotary evaporation yielded a blue solid, 211 mg (87%), mp 192-193°; ^{1}H nmr (deuteriochloroform): δ 11.04 (br t, 1H), 10.89 (br t, 1H), 9.22 (s, 1H), 8.58 (s, 1H), 7.56 (m, 2H), 7.38 (m, 5H), 5.42 (s, 2H), 4.99 (br t, 2H), 3.57 (m, 4H), 3.42 (m, 4H), 1.46 (s, 18H).

Anal. Calcd. for $C_{34}H_{41}N_5O_7$: C, 64.64; H, 6.54; N, 11.09. Found: C, 64.62; H, 6.52; N, 11.00.

4-Benzyloxy-6,9-bis[[2-(dimethylamino)ethyl]amino]benz[*g*]-isoquinoline-5,10-dione (**18b**).

A solution of **4c** (47 mg, 0.134 mmole) and *N,N*-dimethylethylenediamine (118 mg, 1.34 mmoles) in pyridine (0.2 ml) was stirred at room temperature for 74 hours. The pyridine and excess diamine were removed by a gentle stream of nitrogen. Purification of the blue residue by column chromatography (silica gel: 1.75 x 15 cm) by gradient elution with chloroform: methanol [98:2, 96:4, 95:5 (50 ml), 90:10 (100 ml), 70:30 (300 ml)] led to a red and a blue band. The removal of the eluents by rotary evaporation yielded a red solid (13 mg, mono(amino-alkyl)amino substituted regioisomeric mixture) and **18b** as a blue solid, 50 mg (77%), mp 193-195°; ^{1}H nmr (deuteriochloroform): δ 11.01 (t, 1H), 10.99 (t, 1H), 9.27 (s, 1H), 8.57 (s, 1H), 7.62 (m, 2H), 7.39 (m, 2H), 7.29 (m, 1H), 7.20 (m, 2H), 5.37 (s, 2H), 3.47 (m, 4H), 2.64 (m, 4H), 2.35 (s, 6H) 2.33 (s, 6H).

Anal. Calcd. for $C_{28}H_{33}N_5O_3$: C, 68.97; H, 6.82; N, 14.36. Found: C, 68.57; H, 6.72; N, 14.32.

4-*p*-Methoxybenzyloxy-6,9-Bis[[2-(dimethylamino)ethyl]-amino]benz[*g*]isoquinoline-5,10-dione (**18c**).

A solution of *N,N*-dimethylethylenediamine (231 mg, 2.62 mmoles) and **4d** (50 mg, 0.131 mmole) in pyridine (1 ml) was stirred at room temperature for 48 hours. The pyridine and excess diamine were removed by a slow nitrogen stream and the resultant blue solid was placed under reduced pressure for 1 hour. The residue was taken up in dichloromethane (3 ml), the solution washed with water (1 ml) and the aqueous layer extracted with dichloromethane (3 x 4 ml). The combined extracts were dried over sodium sulfate and concentrated by rotary evaporation. The blue solid was purified by column chromatography (silica gel, 2.25 x 15 cm) using gradient elution mixtures of methanol: dichloromethane progressively containing 1%, 2%, 4%, 6%, 8%, 15%, 20%, 25%, 30% methanol followed by 30% methanol: 70% dichloromethane: small amount of aqueous ammonium hydroxide. Upon removal of the eluents from the major blue band, a blue solid was collected, 28 mg (42%), mp 178-179°; ^{1}H nmr (deuteriochloroform): δ 10.98 (t, 1H), 10.81 (t, 1H), 9.29 (s, 1H), 8.59 (s, 1H), 7.52 (d, J_{HH} = 8.5 Hz, 2H), 7.28 (m, 2H), 6.92 (d, J_{HH} = 8.5 Hz, 2H), 5.54 (s, 2H), 3.82 (s, 3H), 3.53 (m, 4H), 2.68 (t, J_{HH} = 6.6 Hz, 4H), 2.38 (s, 6H), 2.36 (s, 6H).

Anal. Calcd. for $C_{29}H_{35}N_5O_4$: C, 67.29; H, 6.81, N, 13.53. Found: 67.20; 6.84; N, 13.43.

4-*p*-Methoxybenzyloxy-6,9-bis[[2-[*N*-*t*-butoxycarbonyl)amino]-ethyl]amino]benz[*g*]isoquinoline-5,10-dione (**18d**).

A solution of **4d** (1.05 g, 2.75 mmoles) and *N*-(*t*-butoxycarbonyl)ethylenediamine (2.2 g, 13.75 mmoles) in *N*-methylpyrrolidinone was stirred at 50° for 16 hours under a nitrogen atmosphere. Upon cooling, the reaction mixture was poured into demineralized water (105 ml) and stirred for 1 hour at 0°. The blue precipitate was collected by filtration, washed thoroughly with water and dried. This material was purified by column chromatography (silica gel, 70-230 mesh, 3 x 20 cm) using as the eluent a mixture of hexane:ethyl acetate (1:1) up to the beginning of the product elution and hexane:ethyl acetate (1:2) up to completion of the elution. Upon removal of the eluents containing the desired product, a blue solid was collected, 1.05 g (58%), mp 89-91°; ^{1}H nmr (deuteriochloroform): δ 11.00 (t, 1H), 10.85 (t, 1H), 9.20 (s, 1H), 8.58 (s, 1H), 7.48 (d, J_{HH} = 8.4 Hz, 2H), 7.35 (br m, 2H), 6.92 (d, J_{HH} = 8.4 Hz, 2H), 5.35 (s, 2H), 5.00 (br s, 2H), 3.83 (s, 3H), 3.57 (br t, 4H), 3.42 (br t, 4H), 1.45 (s, 18H).

Anal. Calcd. for $C_{35}H_{43}N_5O_8$: C, 63.52; H, 6.55; N, 10.58. Found: C, 63.25; H, 6.58; N, 10.45.

O-*p*-Methoxybenzyl-*N,N'*-diisopropylisourea.

Under a nitrogen blanket, *N,N'*-diisopropylcarbodiimide (91.3 g, 0.723 mole) was added to a stirred suspension of copper(II) chloride (235 mg) in 4-methoxyphenylmethanol (100 g, 0.723 mole) cooled to 10-15°. After the addition, the mixture was heated at 60° for 2 hours and stirred at room temperature overnight. The catalyst was removed by filtration and the product was obtained quantitatively as a crude oil which was used without further purification. Attempts to distill the crude material led to partial decomposition; ^{1}H nmr (deuteriochloroform): δ 7.32 (d, J_{HH} = 8.4 Hz, 2H), 6.85 (d, J_{HH} = 8.4 Hz, 2H), 5.04 (s, 2H), 3.85 (s, 3H), 3.4 (m, 1H), 3.2 (m, 1H), 1.15 (d, J_{HH} = 4.5 Hz, 6H), 1.10 (d, J_{HH} = 4.5 Hz, 6H).

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