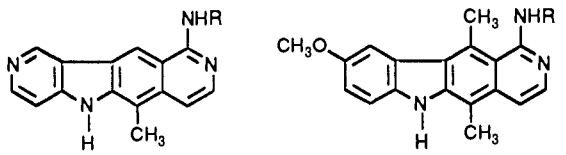
Synthesis and Antitumor Activity of 1-[[(Dialkylamino)alkyl]amino]-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*])indoles. A New Class of Antineoplastic Agents

Chi Hung Nguyen,† Jean-Marc Lhoste,‡ François Lavelle,§ Marie-Christine Bissery,§ and Emile Bisagni*,†

URA 1387 CNRS, Synthèse Organique, U 219 INSERM, Biophysique, Institut Curie, section de Biologie, Bâts. 110-112, 15 rue Georges Clêmenceau, 91405 Orsay, France, and Rhône Poulenc Santé, Centre de Recherches de Vitry, 13 quai Jules Guesde, 94403 Vitry-sur-Seine, France. *Received July 10, 1989*

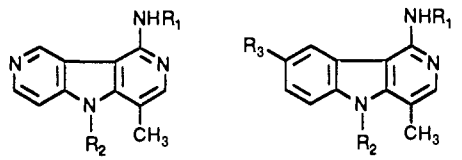
The thermal Fischer indolization of hydrazones resulting from 4-hydrazino-5-methyl-1*H*-pyridin-2-one and various β- and α-tetralones led to 4-methyl-6,7-dihydro-2*H*,5*H*-pyrido[4,3-*b*]benzo[*e*]indol-1-ones and 4-methyl-10,11-dihydro-2*H*,5*H*-pyrido[4,3-*b*]benzo[*g*]indol-1-ones, respectively. After aromatization, these compounds were transformed by phosphorus oxychloride, giving 1-chloro-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*]indoles which were substituted by [(dialkylamino)alkyl]amines. The resulting 1-[[(dialkylamino)alkyl]amino]-4-methyl-5*H*-pyrido-[4,3-*b*]benzo[e]- and -benzo[*g*]indoles, as well as hydroxy derivatives obtained by demethylation of methoxylated compounds with hydrobromic acid, were tested for antitumor activity in vitro (leukemic and solid tumor cells) and in vivo on various experimental tumor models using the standard NCI protocols. 1-[[3-(Dialkylamino)propyl]-amino]-4-methyl-9-hydroxy-5*H*-pyrido[4,3-*b*]benzo[*e*]indoles appeared as a promising new class of antineoplastic agents.

As noticed in the various series, the adjunction of a [(dialkylamino)alkyl]amino side chain on a polycyclic-DNA-intercalating system displaying antitumor properties clearly increased the biological activity. Thus, 10-[[3-(diethylamino)propyl]amino]-6-methyl-5*H*-pyrido-[3′,4′:4,5]pyrrolo[2,3-*g*]isoquinoline (**1**, BD40) and 1-[[3-(diethylamino)propyl]amino]-9-methoxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (**2**, BD84), which were synthesized in this laboratory,[1,2] were highly active antineoplastic compounds[3-5] and are currently undergoing clinical trials. Promising results were obtained in phase I trials with compound **1**.[6]



1 **2**

R = $CH_2CH_2CH_2N(CH_2CH_3)_2$

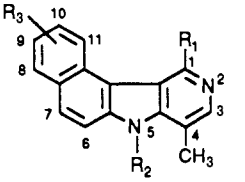


3 **4**

The first results obtained with compounds related to **1** and **2** indicated that DNA intercalation was a necessary but not a sufficient condition to retain activity.[4,5] Further studies were done with the tricyclic series **3**[7,8] and **4**,[9] which correspond to the heterocyclic systems **1** and **2** simplified by deletion of an aromatic ring. For these new compounds and as long as the key substituents R_1, R_2, and R_3 [(i) R_1 = $CH_2CH_2CH_2N(R)_2$ with R = CH_3 or CH_2CH_3, (ii) R_2 = H or CH_3, (iii) R_3 = OH in the series **4**] were present, in vitro cytotoxicities and in vivo antitumor properties were low in series **3**[10] and important for series **4**.[11] DNA affinities of tricyclic derivatives **3** and **4** were lower than those of compounds **1** and **2**.[10,11]

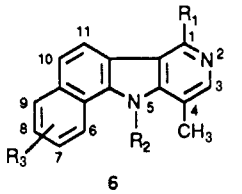
As hypothesized,[7] the 4-CH_3 group was shown to play a central role.[10,11] Therefore, the structure of the chromophore itself seemed to be the determinant for antitumor activity. Thus, it would be interesting to study new compounds related to series **4** with an additional aromatic ring in the angular position. Such a ring seemed a priori able to reestablish a good DNA affinity.

With these considerations in mind, we undertook the synthesis of 5*H*-pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*]indole derivatives corresponding to structures **5** and **6**, trying to keep the key substituents of series **4**.



5

4 - methyl-5*H* - pyrido[4,3-*b*]benzo[*e*]ndoles



6

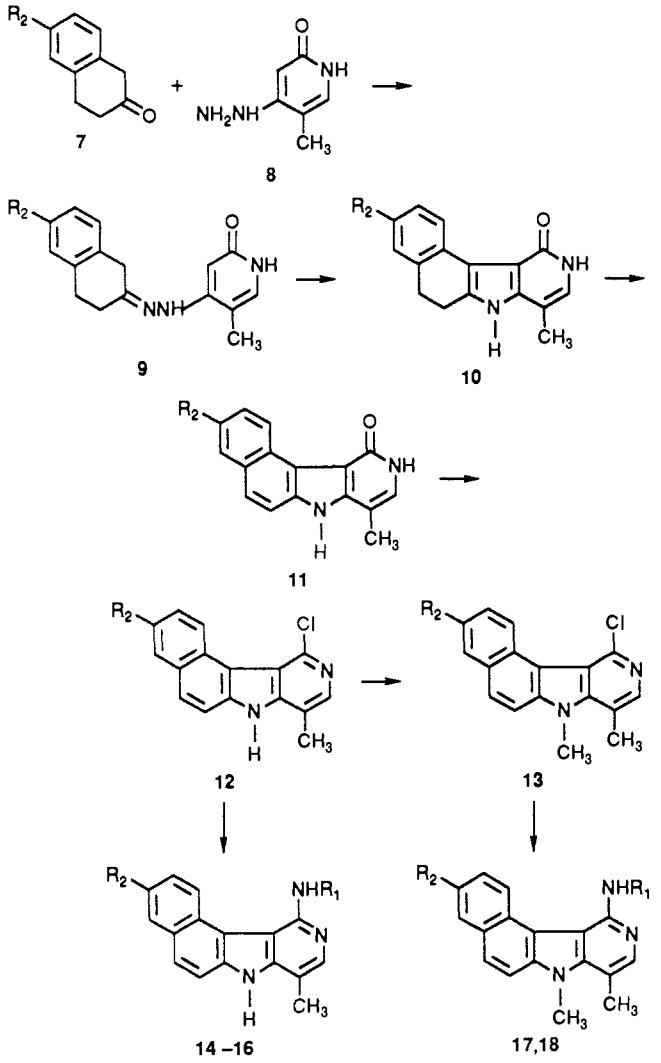
4 - methyl-5*H* - pyrido[4,3-*b*]benzo[*g*]indoles

† Synthèse Organique, Institut Curie.
‡ Biophysique, Institut Curie.
§ Centre de Recherches de Vitry.

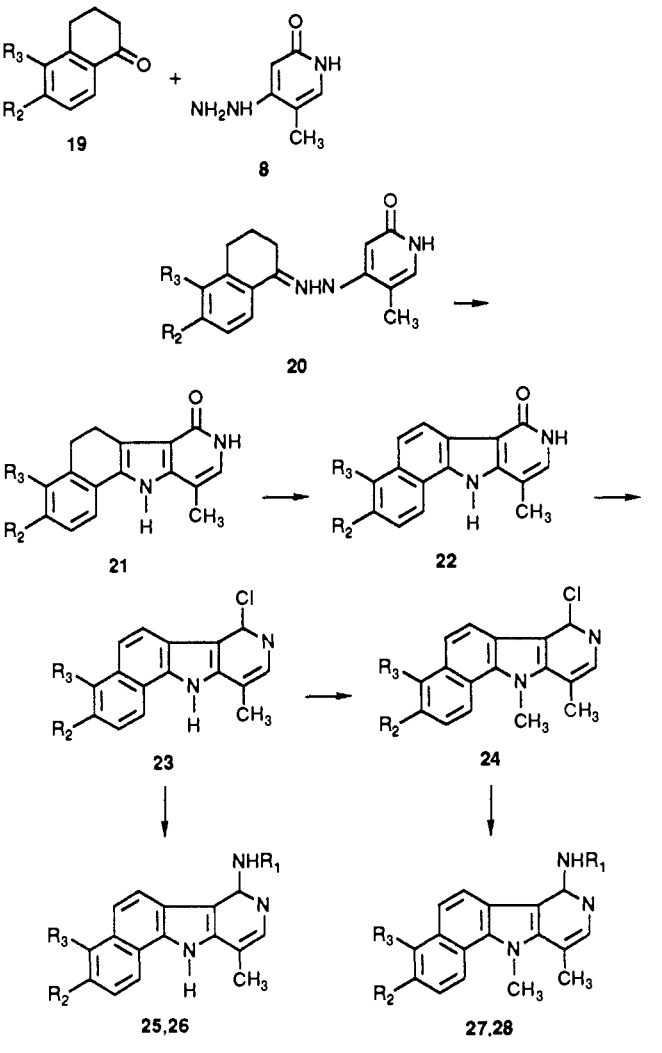
(1) Ducrocq, C.; Bisagni, E.; Rivalle, C.; Lhoste, J.-M. *J. Chem. Soc., Perkin Trans. 1* **1979**, 142.
(2) Bisagni, E.; Ducrocq, C.; Lhoste, J.-M.; Rivalle, C.; Civier, A. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1706.
(3) Lidereau, C.; Chermann, J-C.; Gruest, J.; Montagnier, L.; Ducrocq, C.; Rivalle, C.; Bisagni, E. *Bull. Cancer* **1980**, *67*, 1.
(4) Ducrocq, C.; Wendling, F.; Tourbez-Perrin, M.; Rivalle, C.; Tambourin, P.; Pochon, F.; Bisagni, E.; Chermann, J-C. *J. Med. Chem.* **1980**, *23*, 1212.
(5) Rivalle, C.; Wendling, F.; Tambourin, P.; Lhoste, J-M.; Bisagni, E.; Chermann, J-C. *J. Med. Chem.* **1983**, *26*, 181.
(6) Marty, M.; Jasmin, C.; Pouillard, P.; Gisselbrecht, C.; Gouvenia, G.; Magdelênat, H. *Annu. Meet. Am. Soc. Clin. Oncol.* **1981**, C-108.
(7) Bisagni, E.; Nguyen, C. H. *Tetrahedron* **1986**, *42*, 2311.
(8) Nguyen, C. H.; Bisagni, E. *Tetrahedron* **1986**, *42*, 2303.
(9) Nguyen, C. H.; Bisagni, E. *Tetrahedron* **1987**, *43*, 535.
(10) Nguyen, C. H.; Bisagni, E.; Pépin, O.; Pierrê, A.; De Cointet, P. *J. Med. Chem.* **1987**, *30*, 1642.
(11) Bisagni, E.; Nguyen, C. H.; Pierrê, A.; Pepin, O.; De Cointet, P.; Gros, P. *J. Med. Chem.* **1988**, *31*, 398.

0022-2623/90/1833-1519$02.50/0

Scheme I



Scheme II



In this paper, we report on the synthesis of these two new series and their in vitro and in vivo biological properties.

Chemistry

(A) 4-Methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indoles (5). β-Tetralone (**7a**) and 6-methoxy-1,2,3,4-tetrahydronaphthalene-2-one (**7b**)[12] were condensed with 4-hydrazino-5-methyl-1*H*-pyridin-2-one, which was already described.[8] The resulting hydrazones **9a** and **9b** were submitted to the thermal Fischer indolization, giving 4-methyl-6,7-dihydro-2*H*,5*H*-pyrido[4,3-*b*]benzo[*e*]indole-1-ones (**10a** and **10b**). Palladium on charcoal aromatization of these two compounds led directly to 4-methyl-2*H*,5*H*-pyrido[4,3-*b*]benzo[*e*]indole-1-ones (**11a** and **11b**). These two reactions (indolization and aromatization) were done in the same vessel, each step being controlled to ensure proper completion. This allowed us to obtain **11a** and **11b** with an overall yield of 73 and 75%, respectively.

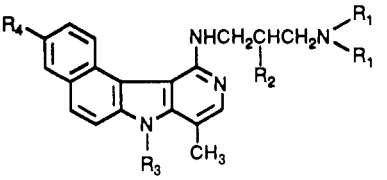
Chlorination of these two compounds was difficult and incomplete in boiling phosphorus oxychloride, even with a large excess of this last compound and a prolonged heating time at reflux. In contrast, 1-chloro-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indoles (**12a** and **12b**) were obtained in excellent yields by boiling **11a** and **11b** in an acetonitrile–phosphorus oxychloride–benzyltriethylammonium chloride mixture, as described by Robins and Uznanski for the chlorination of guanosine triacetate.[13] 5-*N*-methyl derivatives **13a** and **13b** were then prepared by methylation of **12a** and **12b** with methyl iodide, in the presence of anhydrous potassium carbonate, and various 1-chloro-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indoles were easily substituted by 3-(dialkylamino)propylamines to provide the corresponding 1-amino-substituted derivatives **14a,b**, **15a,b**, **16b**, **17a,b**, and **18a,b** in high yields. Finally, demethylation of **14b**, **15b**, **16b**, **17b**, and **18b** was performed with boiling concentrated hydrobromic acid, giving 9-hydroxylated compounds **14c**, **15c**, **16c**, **17c**, and **18c**, respectively (Scheme I).

(B) 4-Methyl-5*H*-pyrido[4,3-*b*]benzo[*g*]indoles (6). These pyrido[4,3-*b*]benzo[*g*]indoles were synthesized starting from α-tetralones (**19**) and hydrazinopyridone 8 via intermediates **20–24** according to a general scheme comparable to the previous one for obtaining benzo[*e*] series **5**.

(12) Nelson, N. A.; Phsi, R. S.; Schuck, J-M.; Kahn, L. D. *J. Am. Chem. Soc.* **1960**, *82*, 2573.

(13) Robins, M. J.; Uznanski, B. *Can. J. Chem.* 1981, *59*, 2601.

Table I. 1-[[3-(Dialkylamino)propyl]amino]-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indoles: In Vitro and in Vivo Biological Results with P388 Leukemia



no.	R_1	R_2	R_3	R_4	ID_{50}, μg/mL	T/C × 100 (optimal daily dose, mg/kg)[a]	survivors at day 50
14a	CH_3	H	H	H	0.1	150 (40)	
14b	CH_3	H	H	OCH_3	0.75	202 (40)	
14c	CH_3	H	H	OH	0.025	270 (10)	
15a	C_2H_5	H	H	H	0.18	149 (40)	
15b	C_2H_5	H	H	OCH_3	1	189 (40)	
15c	C_2H_5	H	H	OH	0.05	215 (10)	
16b[b]	CH_3	CH_3	H	OCH_3	0.55	151 (40)	
16c[b]	CH_3	CH_3	H	OH	0.1	213 (20)	3/5
17a	CH_3	H	CH_3	H	0.05	164 (40)	
17b	CH_3	H	CH_3	OCH_3	0.5	150 (40)	
17c	CH_3	H	CH_3	OH	0.015	243 (20)	1/5
18a	C_2H_5	H	CH_3	H	0.1	124 (40)	
18b	C_2H_5	H	CH_3	OCH_3	0.75	143 (40)	
18c	C_2H_5	H	CH_3	OH	0.025	232 (20)	

[a] Daily dose giving the optimal therapeutic effect, without apparent toxicity (no lethal effect, weight variations <10% of body weight). [b] (±)-Mixture.

The substitutions of chloro derivatives **23** and **24** which led to 1-amino-substituted-4-methyl-5*H*-pyrido[4,3-*b*]-benzo[*g*]indoles **25–28**, as well as the demethylations for obtaining hydroxylated derivatives **c** and **e**, were all performed under the aforementioned conditions for series **5**.

A problem worthy of attention is the difficult chlorination of 4-methyl-2*H*,5*H*-pyrido[4,3-*b*]benzo[*g*]indole-1-ones (**22**). This has been resolved by using the Robins and Uznanski chlorination mixture, which leads to excellent yields of chloro derivatives **23** (Scheme II).

Biological Studies

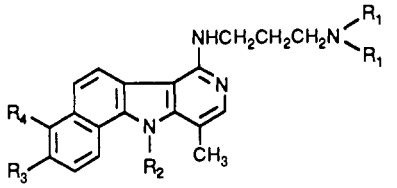
(a) In Vitro Cytotoxicity Determination. Cytotoxicity studies for tumor cells grown in vitro are described in the Experimental Section. The ID_{50} values of all the compounds tested against P388 cells are reported in Tables I and II.

Differential in vitro cytotoxicities were also studied in the multiple tumor disk diffusion soft agar assay, using murine leukemia tumor cells (L1210) and solid tumor cells (colon adenocarcinoma C38 and pancreatic ductal adenocarcinoma O3 P03). Corresponding results are given in Table III. The most significant differentials were obtained with compounds **14c**, **15c**, **16c**, **17c**, and **18c**, all belonging to the 4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indole series.

(b) In Vivo Antitumor Effects. 1-Amino-substituted-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indoles **14–18** and 1-amino-substituted-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*g*]-indoles **25–28** were first tested in vivo against P388 leukemia, according to the protocol of Geran et al.[14] Results are indicated in Tables I and II. The most active compounds were **14b**, T/C = 202%; **14c**, T/C = 270%; **15c**, T/C = 215%; **18c**, T/C = 232%; and especially derivatives **16c**, T/C = 213%; and **17c**, T/C = 243%, with respectively 3/5 and 1/5 survivors on day 50. In addition these compounds were tested on L1210 leukemia. This system is more severe than P388 leukemia and has also been considered to be more predictive for clinical activity.[15] Results of this investigation, done in comparison with compounds **1** (BD40) and **2** (BD84) currently undergoing clinical trials, are reported in Table IV. Compounds **14c** and **16c** were the most active in this system, respective T/C values being 143% (5/8) and 149% (2/8), each with 50-day survivors.

These derivatives were further studied in comparison with reference compounds **1** and **2** in four solid tumor models: mammary adenocarcinoma 16/C (MA 16/C),[16]

Table II.
1-[[3-(Dialkylamino)propyl]amino]-4-methyl-5*H*-pyrido[4,3-*b*]-benzo[*g*]indoles: In Vitro and in Vivo Biological Results with P388 Leukemia



no.	R_1	R_2	R_3	R_4	ID_{50}, μg/mL	T/C × 100 (optimal daily dose, mg/kg)[a]
25a	CH_3	H	H	H	0.375	115 (10)
25d	CH_3	H	H	OCH_3	0.15	121 (20)
25e	CH_3	H	H	OH	0.05	169 (20)
26a	C_2H_5	H	H	H	0.75	102 (10)
26b	C_2H_5	H	OCH_3	H	0.5	131 (40)
27a	CH_3	CH_3	H	H	0.375	119 (10)
27d	CH_3	CH_3	H	OCH_3	0.75	130 (40)
27e	CH_3	CH_3	H	OH	0.025	135 (20)
28a	C_2H_5	CH_3	H	H	0.375	108 (20)
28b	C_2H_5	CH_3	OCH_3	H		
28c	C_2H_5	CH_3	OH	H	0.1	124 (20)
28d	C_2H_5	CH_3	H	OCH_3	0.75	124 (40)
28e	C_2H_5	CH_3	H	OH	0.05	160 (10)

[a] Daily dose giving the optimal therapeutic effect, without apparent toxicity (no lethal effect, weight variations <10% of body weight).

(14) Geran, R. J.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abott, B. J. *Cancer Chemother. Rep. Part 3* **1972**, 3.

(15) Staquet, M. J.; Byar, D. P.; Green, S. B.; Rozencweig, M. *Cancer Treat. Rep.* **1983**, *67*, 753.

Table III. Cytotoxicity in the Multiple Tumor Disk Diffusion Soft Agar Assay

no.	dose, μg/disk	leukemia L1210, U[a]	solid tumors PO3, U	solid tumors C38, U
14a	15	550–675		675
14b	15	750	630	800
	15	350	300	450
14c	15	375	850	700–800
15a	15	420–525		525
15b	ND[b]			
15c	15	475		625
	15	450–650	900	
16b	15	425		425
	15	450–525	375	
16c	15	300	850	675–700
17a	15	425		450–525
	15	475–600	550	
17b	15	425		325
	15	550–680	200	
17c	15	350		750
	15	0–450	350–450	
18a	15	450–600		520
18b	15	400		325
	15	450–500	375	
18c	15	450		650–675
25a	15	500		550–600
	15	420–490	310	
25d	15	280	225	
25e	15	450		400
	15	500	300–450	
26a	15	500		460
	15	300–375	200	
26b	15	200		285
	15	325	200	
27a	15	450		480
	15	500–580	325	
27d	15	375		675
	15	290–350	275	
27e	15	675		900
28a	15	450		500
	15	300–380	200	
28b	ND			
28c	15	300		325
	15	400	350	
28d	15	450		430
28e	15	500		730
	15	450	430–480	

[a] Results are expressed in units of inhibition (U) 1 unit = 32 μm. [b] ND = not determined.

colon adenocarcinoma 26 (C26),[17] Lewis lung carcinoma (3LL),[18] and B16 melanoma (B16).[19] Results are reported in Table V. In all these experiments the site of drug injection (intravenous, iv) was separated from the site of tumor implantation (subcutaneous, sc, or intramuscular, im) to better mimic the clinical situation. Thus, important parameters such as biodisponibility and tissue distribution of the drug could be taken into account.[20] Compound **14c** was found to be highly active on the four tumors tested: MA 16/C, T/C = 2%; C26, T/C = 0%; 3LL, early stage (day 1) T/C = 0%; 3LL, advanced stage (day 7) T/C = 34%; B16, T/C = 28%.

The last point which had to be verified was the contribution of the benzo[e] ring. For this purpose, the antitumor activity of an active drug corresponding to the general structure **5** (**14c**) against P388 leukemia was compared (ip graft, iv treatment) to that of two structurally related active compounds derived from general structure **4**. Corresponding results are reported in Table VI; compound **14c** had a better antitumor activity (T/C = 182%) than tricyclic derivatives **4a** (T/C = 128%) and **4b** (T/C = 130%).

Table IV. Biological Results with L1210 Leukemia

no.	T/C × 100	optimal daily dose,[a] mg/kg	survivors at day 50
14b	127	20	
14c	143	10	5/8
15b	112	40	
15c	149	10	
16c	149	20	2/8
17c	127	20	
18c	129	20	
1 (BD 40)	151	10	
2 (BD 84)	246	10	3/8

[a] Daily dose giving the optimal therapeutic effect, without toxicity (no lethality, weight variations <10% of body weight).

Discussion and Conclusions

The synthesis of 1-amino-substituted-5*H*-pyrido[4,3-*b*]benzo[*e*]indoles (**5**) and 1-amino-substituted-5*H*-pyrido[4,3-*b*]benzo[*g*]indoles (**6**) were undertaken in order to evaluate their cytotoxic and antitumor properties. Comparison with antitumor tricyclic analogues of ellipticines, namely pyrido[4,3-*b*]indoles or γ-carbolines (**4**), could lead to a better knowledge of structure–activity relationships in these series of new antitumor drugs.

On the basis of previous findings in two series of tricyclic systems,[10,11] the extent of the present work was restricted to various ring-substituted 4-methyl-5*H*-pyrido[4,3-*b*]-benzo[*e*]- and -benzo[*g*]indoles with the 1-substituent limited to a [3-(dialkylamino)propyl]amino group.

As was expected, tetracyclic compounds such as derivatives of series **5** and **6** would interact with DNA with an increased affinity, compared to that of tricyclic analogues **4**. However, this parameter, which did not correlate with cytotoxicity in the series **4**, remains to be studied. Therefore, it will not be considered in this discussion and only biological data will be taken into account.

Among the 27 derivatives of series **5** and **6** which have been synthesized and studied in vitro for cytotoxicity on various cultured cells, eight of them showed ID_{50} values with P388 cells at concentrations <0.05 μg/mL (**14c**, **15c**, **17a**, **17c**, **18c**, **25e**, **27e**, and **28e**, Tables I and II). From differential cytotoxicity studies on leukemia murine tumor cells (L1210) and on solid tumors cells (PO3, C38) in the multiple tumor disk diffusion soft agar assay, it was apparent that the 1-amino-substituted-5*H*-pyrido[4,3-*b*]-benzo[*g*]indoles series had no marked specificity. On the contrary, in the benzo[*e*] series, compounds **14c** and **16c** showed preferential cytotoxicity toward murine solid tumor cells. Compound **14c** had a 475 units zone differential toward PO_3 and a 325 units zone differential toward colon 38. Compound **16c** had a 550 units zone differential toward PO3 and a 375 units zone differential toward colon 38. A differential superior to 250 units indicates significant differential activity toward solid tumor cells. Since corresponding 9-H (**14a**) and 9-OCH_3 (**14b**) substituted compounds did not disclose such differential effects, this specificity is obviously correlated with the presence of a hydroxyl group at the 9-position. For all equivalent series, comparison of cytotoxicities in various sytems showed that hydroxy substituents markedly increased biological response, whereas, for equivalent H and OCH_3-substituted

(16) Corbett, T. H.; Griswold, D. P.; Roberts, B. J.; Peckham, J. C.; Schabel, F. M. *Cancer Treat. Rep.* **1978**, *62*, 1471.
(17) Corbett, T. H.; Griswold, D. P.; Roberts, B. J.; Peckham, J. C.; Schabel, F. M. *Cancer Res.* **1975**, *35*, 2434.
(18) Mayo, J. G. *Cancer Chemother. Rep. Part 2* **1972**, *3*, 325.
(19) Goldin, A.; Johnson, R. K.; Venditti, J. M. *Cancer Chemother. Rep. Part 2* **1975**, *5*, 21.
(20) Atassi, G. *Eur. J. Cancer Clin. Oncol.* **1980**, *20*, 699.

Table V. Comparative Activities of Compounds 1, 2, 14c, and 16c in Murine Solid Tumor Models

tumor	implant route	no. of cells	agent	iv treatment: optimal dose, mg/kg per injection	schedule	total	therapeutic response wt,[a] g	% T/C[b] (day)[c]
MA 16/C	sc	fragment (1 mm^3)	1	5.0	1-4	20.0	-1.5	45 (15)
			2	2.5	1-4	10.0	-2.2	4 (15)
			14c	10.0	1-4	40.0	-1.7	2 (15)
			16c	ND[d]				
C26	sc	fragment (1 mm^3)	1	10	1-4	40.0	-1.5	10 (14)
			2	2.5	1-4	10.0	-2.1	0 (14)
			14c	10	1-4	40.0	-1.9	0 (14)
			16c	ND				
B16	sc	fragment (1 mm^3)	1	5.0	1-4	20.0	-0.5	10 (15)
			2	1.25	1-4	5.0	+1.0	26 (15)
			14c	5.0	1-4	20.0	-1.1	28 (15)
			16c	ND				
3LL (early)	im	10^6	1	ND				
			2	2.5	1-4	10.0	-1.4	0 (17)
			14c	10.0	1-4	40.0	-2.1	0 (17)
			16c	20.0	1-4	80.0	-1.7	0 (17)
3LL (advanced)	im	10^6	1	ND				
			2	2.5	7-10	10.0	0	40 (17)
			14c	10.0	7-10	40.0	-2.8	34 (17)
			16c	ND				

[a] Change in body weight between day 1 and 7 for MA 16/C, C26, B16, and 3LL early stage and between day 1 and 14 for 3LL advanced stage. [b] % T/C = medium tumor weight of treated/control animals × 100 for MA 16/C, C26, and B16. % T/C = mean number of metastases in treated/control animals × 100 for 3LL. [c] Day of analysis. [d] ND = not determined.

Table VI. In Vivo Antitumor Activity of Compound 14c, Compared to That of Derivatives of Series 4: P388 Leukemia Ip Graft Iv Treatment

no.	R_1	R_2	R_3	T/C × 100	optimal daily dose,[a] mg/kg
4a	$(CH_2)_3N(CH_3)_2$	CH_3	OH	128	5
4b	$(CH_2)_3N(CH_3)_2$	H	OH	130	2.5
14c				182	5.0

[a] The optimal daily dose corresponds to that one giving the better therapeutic effect without toxicity (no lethality, weight variations <10% of total weight). P388 cells (10^6) were grafted by ip route at day 0 (10 mice/group). Drugs were injected iv, at days 1, 2, 3, and 4, in a volume of 10 mL/kg. Antitumor activity is expressed by the T/C × 100 value.

Table VII. Hydrazones 9a, 9b, 20a, 20b, and 20d: Experimental Conditions and Physical Data

no.	heating time, h	% yield	mp, °C	formula	anal.
9a	6	77	257-260 dec	$C_{16}H_{17}N_3O$	CHN
9b	4.5	98	240-250 dec	$C_{17}H_{19}N_3O_2 \cdot 0.25H_2O$	CHN
20a	18	89	>270	$C_{16}H_{17}N_3O$	CHN
20b	48	86	270	$C_{17}H_{19}N_3O_2 \cdot 2H_2O$	CHN
20d	15	90	>270	$C_{17}H_{19}N_3O_2$	CHN[a]

[a] Calcd: C, 68.66; H, 6.44; N, 14.13. Found: C, 68.46; H, 6.99; N, 14.44.

derivatives, cytotoxicities were either lowered or abolished. This was particularly true in the 1-[[3-(dimethylamino)-propyl]amino]-4-methyl-9-substituted-5*H*-pyrido[4,3-*b*]-benzo[*e*]indole series. Concerning the 1-dibasic side chain, three kinds of 3-(dialkylamino)propylamino derivatives have been studied; the [3-(dimethylamino)propyl]amino group always gave the most pronounced cytotoxic effects.

The in vivo antitumor effects on P388 leukemia are reported in Tables I and II. All 1-amino-substituted-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indole derivatives **14–18** exhibited various degrees of antitumor activity, except compounds **15a**, **18a**, and **18b**. In contrast, among compounds **25–28**, all derived from the benzo[*g*] series, only **25c** and **28c** had marginal, but significant, activity. These results correlated well with in vitro observations. The most significant in vivo P388 active compounds were **14b**, **14c**, **15b**, **18c**, and especially **16c** and **17c**, which gave long-term survivors. The activity of these six compounds was evaluated in the more severe L1210 leukemia model. Compounds **14c** and **16c** appeared to be the most active ones (with long term survivors) compared with reference compounds **1** and **2** (Table IV). Comparison of **14c** and **16c** with reference compounds **1** and **2** was also performed on solid tumor models. As can be pointed out in Table V, 1-[[3-(dimethylamino)propyl]amino]-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indole (**14c**) shows important anti-

Table VIII. 2*H*,5*H*-Pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*]indol-1-ones 11a,b and 22a,b,d: Physical Data

no.	% yield	mp, °C	formula	anal.	^1H NMR [$(CD_3)_2SO$], δ (ppm)
11a	73	>270	$C_{16}H_{12}N_2O$	CHN	2.35 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 0.8 Hz), 7.14 (m, 1 H, 3-H), 7.36–7.68 (m, 2 H, 9-H + 10-H), 7.68–7.92 (m, 2 H, 6-H + 7-H), 7.92–8.05 (m, 1 H, 8-H), 10.38 (d × d, 1 H, 11-H, $J_{11\text{-}10}$ = 8 Hz, $J_{11\text{-}9}$ = 1.8 Hz), 10.93 (br s, 1 H, 2-NH), 12.08 (br s, 1 H, 5-NH)
11b	75	260	$C_{17}H_{14}N_2O_2 \cdot 0.25H_2O$	CHN	2.34 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 0.7 Hz), 3.92 (s, 3 H, OCH_3), 7.10 (d, 1 H, 3-H), 7.23 (d × d, 1 H, 10-H, $J_{10\text{-}11}$ = 9.2 Hz, $J_{10\text{-}8}$ = 2.7 Hz), 7.42 (d, 1 H, 8-H), 7.73 (s, 2 H, 6-H + 7-H), 10.33 (d, 1 H, 11-H), 10.93 (br s, 1 H, 2-NH), 12.04 (s, 1 H, 5-N*H*)
22a	95	>270	$C_{16}H_{12}N_2O$	CHN	2.42 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 1 Hz), 7.12 (m, 1 H, 3-H), 7.4–7.8 (m, 3 H, 8-H + 7-H + 10-H), 8.04 (d × d, 1 H, 9-H, $J_{9\text{-}8}$ = 7.8 Hz, $J_{9\text{-}7}$ = 1.7 Hz), 8.30 (d, 1 H, 11-H, $J_{11\text{-}10}$ = 8.5 Hz), 8.6–8.8 (m, 1 H, 6-H, $J_{6\text{-}10}$ = 8.5 Hz), 10.95 (br s, 1 H, 2-NH), 12.24 (br s, 1 H, 5-NH)
22b	70	>270	$C_{17}H_{14}N_2O_2 \cdot 0.5H_2O$	CHN	2.40 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 0.9 Hz), 3.94 (s, 3 H, OCH_3), 7.10 (br s, 1 H, 3-H), 7.34 (d × d, 1 H, 7-H, $J_{7\text{-}6}$ = 9 Hz, $J_{7\text{-}9}$ = 3 Hz), 7.50 (d, 1 H, 9-H), 7.64 (d, 1 H, 10-H, $J_{10\text{-}11}$ = 8.4 Hz), 8.27 (d, 1 H, 11-H), 8.63 (d, 1 H, 6-H), 10.94 (br s, 1 H, 2-NH), 12.15 (br s, 1 H, 5-NH)
22d	78	>270	$C_{17}H_{14}N_2O_2$	CHN	2.36 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 1 Hz), 4.0 (s, 3 H, OCH_3), 7.0 (d, 1 H, 8-H, $J_{8\text{-}7}$ = 8.1 Hz), 7.13 (m, 1 H, 3-H), 7.55 (t, 1 H, 7-H, $J_{7\text{-}6}$ = 8.1 Hz), 8.0 (d, 1 H, 10-H, $J_{10\text{-}11}$ = 8.7 Hz), 8.2–8.4 (m, 2 H, 11-H + 6-H), 10.9 (br s, 1 H, 2-NH), 12.1 (br s, 1 H, 5-NH)

Table IX. 1-Chloro-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*]indoles **12a,b** and **23a,b,d**: Physical Data

no.	% yield	mp, °C	formula	anal.	^{1}H NMR [$(CD_3)_2SO$], δ (ppm)
12a	82	>270	$C_{16}H_{11}ClN_2$	CHNCl[a]	2.61 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 0.9 Hz), 7.40–7.90 (m, 3 H, 9-H + 10-H + 6-H), 8.0–8.16 (m, 3 H, 3-H + 7-H + 8-H), 9.60–9.78 (m, 1 H, 11-H, $J_{11\text{-}10}$ = 8.6 Hz), 12.54 (br s, 1 H, 5-NH)
12b	92	>270	$C_{17}H_{13}ClN_2O$	CHNCl	2.60 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 0.7 Hz), 3.95 (s, 3 H, OCH_3), 7.39 (d × d, 1 H, 10-H, $J_{10\text{-}11}$ = 9.2 Hz, $J_{10\text{-}8}$ = 2.8 Hz), 7.58 (d, 1 H, 8-H), 7.83 (d, 1 H, 6-H, $J_{6\text{-}7}$ = 8.9 Hz), 8.05 (d, 1 H, 7-H), 8.10 (q, 1 H, 3-H), 9.67 (d, 1 H, 11-H), 12.49 (s, 1 H, 5-NH)
23a	70	>270	$C_{16}H_{11}ClN_2$	CHNCl	2.69 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 1 Hz), 7.5–7.9 (m, 3 H, 7-H + 8-H + 10-H), 8.0–8.2 (m, 2 H, 3-H + 9-H), 8.48 (d, 1 H, 11-H, $J_{11\text{-}10}$ = 8.7 Hz), 8.7–8.9 (m, 1 H, 6-H), 12.59 (br s, 1 H, 5-NH)
23b	70	>270	$C_{17}H_{13}ClN_2O$	CHNCl	2.66 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 0.8 Hz), 3.97 (s, 3 H, OCH_3), 7.41 (d × d, 1 H, H-7, $J_{7\text{-}6}$ = 9 Hz, $J_{7\text{-}9}$ = 2.5 Hz), 7.57 (d, 1 H, 9-H), 7.75 (d, 1 H, 10-H, $J_{10\text{-}11}$ = 8.7 Hz), 8.55 (m, 1 H, 3-H), 8.41 (d, 1 H, 11-H), 8.72 (d, 1 H, 6-H), 12.45 (br s, 1 H, 5-NH)
23d	71	>270	$C_{17}H_{13}ClN_2O$	CHNCl	2.67 (s, 3 H, 4-CH_3), 4.07 (s, 3 H, OCH_3), 7.18 (d × d, 1 H, 8-H, $J_{8\text{-}7}$ = 7.8 Hz, $J_{8\text{-}6}$ = 0.6 Hz), 7.5–7.8 (m, 1 H, 7-H), 8.0–8.2 (m, 2 H, 3-H + 10-H), 8.3–8.5 (m, 2 H, 6-H + 11-H, $J_{11\text{-}10}$ = 9 Hz), 12.51 (br s, 1 H, 5-NH)

[a] Calcd: C, 72.04; H, 4.13; N, 10.51; Cl, 13.32. Found: C, 71.76; H, 4.14; N, 10.70; Cl, 13.96.

Table X. 1-Chloro-4,5-dimethyl-5*H*-pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*]indoles **13a,b** and **24a,b,d** Physical Data

no.	% yield	mp, °C	formula	anal.	^{1}H NMR [$(CD_3)_2SO$], δ (ppm)
13a	91	167–168	$C_{17}H_{13}ClN_2$	CHNCl	2.85 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 0.9 Hz), 4.30 (s, 3 H, N-CH_3), 7.40–7.80 (m, 2 H, 9-H + 10-H), 7.80–8.20 (m, 4 H, 6-H + 3-H + 8-H + 7-H, $J_{6\text{-}7}$ = 9 Hz), 9.45–9.60 (m, 1 H, 11-H, $J_{11\text{-}10}$ = 8.8 Hz)
13b	86	185–187	$C_{18}H_{15}ClN_2O$	CHNCl	2.86 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 0.7 Hz), 3.95 (s, 3 H, OCH_3), 4.31 (s, 3 H, NCH_3), 7.38 (d × d, 1 H, 10-H, $J_{10\text{-}11}$ = 9.3 Hz, $J_{10\text{-}8}$ = 2.7 Hz), 7.59 (d, 1 H, 8-H), 7.99 (d, 1 H, 6-H, $J_{6\text{-}7}$ = 9.1 Hz), 8.05 (m, 1 H, 3-H), 8.12 (d, 1 H, 7-H), 9.49 (d, 1 H, 11-H)
24a	76	162–164	$C_{17}H_{13}ClN_2$	CHNCl	2.87 (d, 3 H, 4-CH_3, $J_{CH_3\text{-}3\text{-}H}$ = 1 Hz), 4.59 (s, 3 H, NCH_3), 7.6–8.0 (m, 3 H, 7-H + 8-H + 10-H), 8.09 (q, 1 H, 3-H), 8.15–8.3 (m, 1 H, 9-H), 8.58 (d, 1 H, 11-H, $J_{11\text{-}10}$ = 8.9 Hz), 8.7–8.9 (m, 1 H, 6-H)
24b	63	166–168	$C_{18}H_{15}ClN_2O$	CHNCl	2.80 (s, 3 H, 4-CH_3), 3.97 (s, 3 H, OCH_3), 4.44 (s, 3 H, NCH_3), 7.31 (d × d, 1 H, 7-H, $J_{7\text{-}6}$ = 9 Hz, $J_{7\text{-}9}$ = 2.4 Hz), 7.56 (d, 1 H, 9-H), 7.73 (d, 1 H, 10-H, $J_{10\text{-}11}$ = 8.4 Hz), 8.0 (s, 1 H, 3-H), 8.45 (d, 1 H, 11-H), 8.59 (d, 1 H, 6-H)
24d	54	224–226	$C_{18}H_{15}ClN_2O$	CHNCl	2.86 (s, 3 H, 4-CH_3), 4.09 (s, 3 H, OCH_3), 4.58 (s, 3 H, NCH_3), 7.22 (d, 1 H, 8-H, $J_{8\text{-}7}$ = 7.9 Hz), 7.5–7.8 (m, 1 H, 7-H), 8.1 (s, 1 H, 3-H), 8.2 (d, 1 H, 10-H, $J_{10\text{-}11}$ = 9 Hz), 8.36 (d, 1 H, 6-H, $J_{6\text{-}7}$ = 8.5 Hz), 8.58 (d, 1 H, 11-H)

tumor activity in these models. At optimal dosage, compound **14c** had T/C values of <10% on MA 16/C, C26, and on early 3LL, indicating high antitumor activity. This level of activity justifies further development by NCI standards.[14]

A final comparison was done with tricyclic (γ-carboline) derivatives, in order to show the contribution of the additional benzo[*e*] ring with respect to simplified ellipticine analogues. Tetracyclic derivatives **14c** was more active in the P388 leukemia model (T/C = 182%, ip graft, iv treatment) than were the most active compounds **4a** and **4b** in the series of γ-carbolines[11] (T/C = 128% for **4a** and 130% for **4b**, Table VI).

In conclusion, among 27 examples of pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*]indoles studied, it was apparent that an additional benzo ring in the *g*-position of pyrido[4,3-*b*]indoles absolishes the antitumor properties. On the other hand, a fused benzo ring in the *e*-position leads to compounds with improved antitumor activity, giving a new series of polycyclic antineoplastic agents: the 1-[[3-(dialkylamino)propyl]amino]-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indoles. Among various compounds of this nature which have been studied, the most promising one is 1-[[3-(dimethylamino)propyl]amino]-9-hydroxy-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indole (**14c**).

Experimental Section

Chemistry. All melting points are uncorrected and were determined with a kofler apparatus. ^{1}H NMR spectra were recorded in the given solvents with a Varian XL 100 apparatus. Purification of products was followed by thin-layer chromatography on silica gel and alumina and further details of the compounds are noted in Tables VII–XII. Elemental analysis was performed by Service Central de microanalyses du CNRS, 91190 Gif-sur-Yvette, France. They are within ±0.4% of the theoretical values corresponding to the mentioned empirical formulas, except for cases which are footnoted.

Hydrazones 9a,b and 20a,b,d. General Procedure. The mixture of 4-hydrazino-5-methyl-1*H*-pyrid-2-ones (13.9 g, 0.1 mol)[8] and the required tetralone (0.12 mol) in dry ethanol (200 mL) was heated at reflux until the disappearance of hydrazinopyridone **8**, as determined by thin-layer chromatography (TLC) on silica gel plates and elution with a methylene chloride–ethanol mixture (9/1, v/v) (Table VII, heating time entry). The resulting hydrazones, which were almost insoluble in ethanol, were purified by boiling in this solvent, giving pale-yellow microcrystals (Table VII).

Transformations of Hydrazones 9a,b and 20a,b,d into 2*H*,5*H*-Pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*]indol-1-ones 11a,b and 22a,b,d. General Procedure. The mixture of the required hydrazone (25 mmol) in diphenyl ether (200 mL) was stirred under N_2 and heated under reflux for 40 min. The mixture was allowed to cool to 200 °C, the disappearance of the starting hydrazone was determined by TLC (conditions indicated for hydrazones), and 10% palladium on charcoal (1.5 g) suspended in diphenyl ether (20 mL) was added cautiously. Except for the **20d** → **22d** transformation, where heating was pursued for a 2.5 h period, the new mixture was heated under reflux for 45 min (disappearance of dihydro intermediate compound, TLC monitoring). Hexane (350 mL) was added to the cooled mixture and the resulting precipitate was filtered and washed with hexane. It was taken up in boiling acetic acid and filtered for elimination of palladized charcoal, which was washed with acetic acid, and

Table XI. 1-[[3-(Dialkylamino)propyl]amino]-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]indoles 14–18: Physical Data

no.	rfx[a]	salt[b]	% yield	mp, °C	formula	anal.	^{1}H NMR (solvent), δ (ppm)
14a	48	2M	45	164–168	$C_{21}H_{24}N_4 \cdot 2C_4H_4O_4$	CHN	[$(CD_3)_2SO$] 2.0–2.25 (m, 2 H, β-CH_2), 2.90 (s, 6 H, $N(CH_3)_2$), 3.15–3.40 (m, 2 H, γ-CH_2), 3.45–3.75 (m, 2 H, α-CH_2), 6.10 (s, 4 H, CH = CH-maleate), 7.45–7.90 (m, 4 H, 9-H + 10-H + 3-H + 6-H, J_{6-7} = 8.6 Hz), 8.0–8.2 (m, 2 H, 7-H + 8-H), 8.80 (d, 1 H, 11-H, J_{11-10} = 8.6 Hz), 12.8 (br s, 1 H, NH); NB: Protons of 4-CH_3 are overlapped by $(CD_3)_2SO$ signals
14b	48	3S	95	200–204	$C_{22}H_{26}N_4O \cdot 3CH_3SO_3H$	CHNS[c]	(D_2O) 1.97–2.38 (m, 2 H, β-CH_2), 2.44 (s, 3 H, 4-CH_3), 2.93 (s, 9 H, $3CH_3SO_3^-$), 3.12 (s, 6 H, $N(CH_3)_2$), 3.15–3.50 (m, 2 × 2 H, γ-CH_2 + α-CH_2), 4.14 (s, 3 H, OCH_3), 7.2–7.5 (m, 4 H, 10-H + 8-H + 3-H + 6-H, J_{8-10} = 2.5 Hz), 7.73 (d, 1 H, 7-H, J_{7-6} = 9.5 Hz), 7.88 (d, 1 H, 11-H, J_{11-10} = 9.5 Hz)
14c	3	2S	95	245–255	$C_{21}H_{24}N_4O \cdot 2CH_3SO_3H \cdot 1.5H_2O$	CHNS	[$(CD_3)_2SO$] 1.70–2.05 (m, 2 H, β-CH_2), 2.16 (s, 6 H, $N(CH_3)_2$), 2.43 (d, 3 H, 4-CH_3, J_{CH_3-3-H} = 0.9 Hz), 3.37–3.68 (m, 2 H, α-CH_2), 6.03 (t, 1 H, N*H*CH_2), 7.21 (d × d, 1 H, 10-H, J_{10-11} = 9 Hz, J_{10-8} = 2.4 Hz), 7.31 (d, 1 H, 8-H), 7.68 (s, 2 H, 6-H + 7-H), 7.76 (q, 1 H, 3-H), 9.09 (d, 1 H, 11-H), 9.44 (br s, 1 H, OH), 11.69 (br s, 1 H, 5-NH); NB: γ-CH_2 signals are overlapped by $(CD_3)_2SO$
15a	24	2M	60	110–120	$C_{23}H_{28}N_4 \cdot 2C_4H_4O_4 \cdot 2H_2O$	CHN	(D_2O) 0.95 (t, 6 H, $(CH_2CH_3)_2$), 1.4–1.8 (m, 2 H, β-CH_2), 1.89 (d, 3 H, 4-CH_3, $J_{CH3-3-H}$ = 0.9 Hz), 2.5–3.0 (m, 8 H, γ-CH_2 + α-CH_2 + $(CH_2CH_3)_2$), 5.7 (s, 4 H, CH = CH-maleate), 6.84 (d, 1 H, 3-H), 7.02 (d, 1 H, 6-H, J_{6-7} = 8.7 Hz), 7.1–7.3 (m, 2 H, 9-H + 10-H), 7.40 (d, 1 H, 7-H), 7.5–7.7 (m, 2 H, 8-H + 11-H)
15b	12	2M	88	211–213	$C_{24}H_{30}N_4O \cdot 2C_4H_4O_4$	CHN	[$(CD_3)_2SO$] 1.28 (t, 6 H, $(CH_3CH_2)_2$), 2.02–2.36 (m, 2 H, β-CH_2), 3.06–3.82 (m, 4 × 2 H, $(CH_2CH_3)_2$ + γ-CH_2 + α-CH_2), 3.97 (s, 3 H, OCH_3), 6.09 (s, 4 H, CH = CH-maleate), 7.39 (d × d, 1 H, 10-H, J_{10-11} = 9.2 Hz, J_{10-8} = 2.3 Hz), 7.63 (d, 1 H, 8-H), 7.75–7.92 (m, 3 H, 6-H + 3-H + 1-NH), 8.02 (d, 1 H, 7-H, J_{7-6} = 8.8 Hz), 8.79 (d, 1 H, 11-H), 12.75 (s, 1 H, 5-NH); NB: 4-CH_3 is overlapped by $(CD_3)_2SO$ signals
15c	2,5	2S	82	254–258	$C_{23}H_{28}N_4O \cdot 2CH_3SO_3H \cdot 4H_2O$	CHNS	[$(CD_3)_2SO$] 1.30 (t, 6 H, $(CH_3CH_2)_2$), 2.0–2.3 (m, 2 H, β-CH_2), 2.40 (s, 3 H, 4-CH_3), 3.0–3.9 (m, 14 H, γ-CH_2 + $(CH_2CH_3)_2$ + $2CH_3SO_3^-$ + α-CH_2), 7.30 (d × d, 1 H, 10-H, J_{10-11} = 9.9 Hz), 7.43 (d, 1 H, 8-H), 7.7–8.0 (m, 3 H, 6-H + 3-H + 7-H), 8.1–8.3 (m, 1 H, 1-NH), 8.45 (d, 1 H, 11-H), 9.24 (br s, 1 H, H^+), 9.76 (br s, 1 H, H^+), 11.61 (s, 1 H, O*H*), 11.9 (br s, 1 H, 5-NH)
16b[d]	24	2M	40	180	$C_{23}H_{28}N_4O \cdot 2C_4H_4O_4$	CHN	(D_2O) 1.11 (d, 3 H, CH_3-β-CH, J_{CH_3-CH} = 6.3 Hz), 2.06 (s, 6 H, $N(CH_3)_2$), 2.12–2.44 (m, 3 H, γ-CH_2 + $CHCH_3$), 2.49 (d, 3 H, 4-CH_3, J_{CH_3-3-H} = 0.9 Hz), 3.42–3.64 (m, 2 H, α-CH_2), 3.86 (s, 3 H, OCH_3), 6.02 (s, 4 H, CH = CH-maleate), 7.29 (d × d, 1 H, 10-H, J_{10-11} = 9 Hz, J_{10-8} = 2.5 Hz), 7.46 (d, 1 H, 8-H), 7.46–7.90 (m, 3 H, 6-H + 3-H + 7-H, J_{6-7} = 8.5 Hz), 9.08 (d, 1 H, 11-H)
16c[d,f]	4	2M	75	215–220	$C_{22}H_{26}N_4O \cdot 2C_4H_4O_4$	CHN	(D_2O) 1.32 (d, 3 H, CH_3-β-CH, J_{CH_3-CH} = 6.5 Hz), 2.41 (m, 4 H, 4-CH_3 + $CHCH_3$), 3.00 (s, 6 H, $N(CH_3)_2$), 3.06–3.32 (m, 4 H, γ-CH_2 + α-CH_2), 6.15 (s, 4 H, CH = CH-maleate), 7.09 (d × d, 1 H, 10-H, J_{10-11} = 9 Hz, J_{10-8} = 2.4 Hz), 7.25 (d, 1 H, 8-H), 7.31–7.67 (m, 3 H, 6-H + 7-H + 3-H, J_{6-7} = 7.4 Hz), 7.79 (d, 1 H, 11-H)
17a	72	2M	42	140–145	$C_{22}H_{26}N_4 \cdot 2C_4H_4O_4 \cdot 1.5H_2O$	CHN	(D_2O) 1.5–1.9 (m, 2 H, β-CH_2), 2.20 (d, 3 H, 4-CH_3, J_{CH_3-3-H} = 1 Hz), 2.58 (s, 6 H, $N(CH_3)_2$), 2.8–3.0 (m, 4 H, γ-CH_2 + α-CH_2), 3.57 (s, 3 H, 5-NCH_3), 5.7 (s, 4 H, CH = CH-maleate), 6.9 (d, 1 H, 3-H), 7.1–7.4 (m, 3 H, 6-H + 9-H + 10-H, J_{6-7} = 8.5 Hz), 7.45–7.75 (m, 3 H, 7-H + 8-H + 11-H)
17b	24	3S	86	115–125	$C_{23}H_{28}N_4O \cdot 3CH_3SO_3H \cdot 2H_2O$	CHNS[e]	(D_2O) 1.53–1.93 (m, 2 H, β-CH_2), 2.25 (d, 3 H, 4-CH_3, J_{CH_3-3-H} = 0.9 Hz), 2.43 (s, 9 H, 3 $CH_3SO_3^-$), 2.61 (s, 6 H, $N(CH_3)_2$), 2.78–3.05 (m, 4 H, γ-CH_2 + α-CH_2), 3.54 (s, 3 H, 5-NCH_3), 3.64 (s, 3 H, OCH_3), 6.84 (d × d, 1 H, 10-H, J_{10-11} = 9 Hz, J_{10-8} = 2.8 Hz), 6.93 (d, 1 H, 8-H), 6.98 (q, 1 H, 3-H), 7.06 (d, 1 H, 6-H, J_{6-7} = 9.1 Hz), 7.31 (d, 1 H, 7-H), 7.49 (d, 1 H, 11-H)
17c	2,5	2S	95	135–150	$C_{22}H_{26}N_4O \cdot 2CH_3SO_3H \cdot 1.5H_2O$	CHNS	[$(CD_3)_2SO$] 2.1–2.35 (m, 2 H, β-CH_2), 2.78 (s, 3 H, 4-CH_3), 2.89 (s, 6 H, $N(CH_3)_2$), 3.15–3.7 (m, 10 H, γ-CH_2 + α-CH_2 + 2 $CH_3SO_3^-$), 4.32 (s, 3 H, 5-NCH_3), 7.2–7.5 (m, 2 H, 10-H + 8-H), 7.7 (br s, 1 H, 3-H), 7.97 (br s, 2 H, 6-H + 7-H), 8.1–8.3 (m, 1 H, NH), 8.56 (d, 1 H, 11-H, J_{11-10} = 9 Hz), 12.7 (br s, 1 H, OH)
18a	24	3HCl	76	250–260	$C_{24}H_{30}N_4 \cdot 3HCl \cdot 2H_2O$	CHNCl	(D_2O) 0.98 (t, 2 × 3 H, $(CH_2CH_3)_2$), 1.45–1.85 (m, 2 H, β-CH_2), 2.19 (s, 3 H, 4-CH_3), 2.7–3.0 (m, 8 H, γ-CH_2 + α-CH_2 + $(CH_2CH_3)_2$), 3.51 (s, 3 H, NCH_3), 6.91 (s, 1 H, 3-H), 7.0–7.4 (m, 3 H, 6-H + 9-H + 10-H), 7.4–7.7 (m, 3 H, 7-H + 8-H + 11-H)
18b	18	2M	72	104–106	$C_{25}H_{32}N_4O \cdot 2C_4H_4O_4 \cdot H_2O$	CHN	[$(CD_3)_2SO$] 0.84 (t, 2 × 3 H, $(CH_3)_2$), 1.74–2.0 (m, 2 H, β-CH_2), 2.32–2.62 (m, 3 × 2 H, $(CH_2CH_3)_2$ + γ-CH_2), 2.70 (d, 3 H, 4-CH_3, J_{CH_3-3-H} = 0.9 Hz), 3.40–3.64 (m, 2 H, α-CH_2), 3.94 (s, 3 H, OCH_3), 4.21 (s, 3 H, NCH_3), 6.05 (br s, 1 H, 1-NH), 7.26 (d × d, 1 H, 10-H, J_{10-11} = 9.3 Hz, J_{10-8} = 2.8 Hz), 7.53 (d, 1 H, 8-H), 7.74 (q, 1 H, 3-H), 7.89 (s, 2 H, 6-H + 7-H), 9.18 (d, 1 H, 11-H)
18c	2,5	2S	76	145–160	$C_{24}H_{30}N_4O \cdot 2CH_3SO_3H \cdot 2H_2O$	CHNS	(D_2O) 1.47 (t, 2 × 3 H, $(CH_3CH_2)_2$, 1.98–2.38 (m, 2 H, β-CH_2), 2.76 (d, 3 H, 4-CH_3, J_{CH_3-3-H} = 0.9 Hz), 2.92 (s, 2 × 3 H, $(CH_3SO_3^-)_2$), 3.30–3.60 (m, 4 × 2 H, $(CH_2CH_3)_2$ + γ-CH_2 + α-CH_2), 4.06 (s, 3 H, NCH_3), 7.28 (d × d, 1 H, 10-H, J_{10-11} = 9.1 Hz, J_{10-8} = 2.4 Hz), 7.38 (d, 1 H, 8-H), 7.50 (q, 1 H, 3-H), 7.58 (d, 1 H, 6-H, J_{6-7} = 9.6 Hz), 7.79 (d, 1 H, 7-H), 7.98 (d, 1 H, 11-H)

[a] Rfx = heating times in hours. [b] M = maleate, S = methanesulfonate. [c] Calcd: C, 46.15; H, 5.84; N, 8.61; S, 14.76. Found: C, 47.21; H, 5.89; N, 8.05; S, 14.54. [d] (±)-Mixture. [e] Calcd: C, 44.57; H, 6.29; N, 8.00; S, 13.72. Found: C, 45.05; H, 6.27; N, 8.04; S, 13.67. [f] Further purification was needed.

the solvent was concentrated. After cooling, the solid was collected, washed with acetone, and air-dried to provide pale-yellow microcrystals. The yields reported in Table VIII were calculated for the compounds obtained under these conditions. However, analytical samples were recrystallized from ethanol in which they are slightly soluble (Table VIII).

Table XII. 1-[[3-(Dialkylamino)propyl]amino]-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*g*]indoles **25–28**: Physical Data

no.	rfx[a]	salt[b]	% yield	mp, °C	formula	anal.	^{1}H NMR (solvent), δ (ppm)
25a	15 d	2M	51.6	175–180	$C_{21}H_{24}N_4 \cdot 2C_4H_4O_4 \cdot 2H_2O$	CHN	(D_2O) 2.0–2.3 (m, 5 H, β-CH_2 + 4-CH_3), 3.02 (s, 6 H, N(CH_3)$_2$), 3.1–3.5 (m, 2 × 2 H, γ-CH_2 + α-CH_2), 6.11 (s, 4 H, CH = CH-maleate), 6.87 (m, 1 H, 3-H), 7.4–7.6 (m, 4 H, 10-H + 11-H + 7-H + 8-H), 7.7–8.0 (m, 2 H, 6-H + 9-H)
25d	24 h[c]	2M	43	210–215 dec	$C_{22}H_{26}N_4O \cdot 2C_4H_4O_4 \cdot 1.5H_2O$	CHN	(D_2O) 1.8–1.95 (m, 5 H, β-CH_2 + 4-CH_3), 2.68 (s, 6 H, N(CH_3)$_2$), 3.0–3.15 (m, 4 H, γ-CH_2 + α-CH_2), 3.68 (s, 3 H, OCH_3), 5.80 (s, 4 H, CH = CH-maleate), 6.45–6.55 (m, 1 H, 8-H), 6.60 (br s, 1 H, 3-H), 7.05–7.15 (m, 3 H, 10-H + 6-H + 7-H), 7.23 (d, 1 H, 11-H, J_{11-10} = 8.9 Hz)
24e[e]	5 h	2M	64.5	200 dec	$C_{21}H_{24}N_4O \cdot 2C_4H_4O_4 \cdot H_2O$	CHN	[(CD_3)$_2$SO] 1.9–2.3 (m, 2 H, β-CH_2), 2.60 (s, 3 H, 4-CH_3), 2.85 (s, 6 H, N(CH_3)$_2$), 3.1–3.35 (m, 2 H, γ-CH_2), 3.55–3.9 (m, 2 H, α-CH_2), 6.1 (s, 4 H, CH = CH-maleate), 7.05 (d, 1 H, 8-H, J_{8-7} = 7.3 Hz), 7.45–7.7 (m, 1 H, 7-H), 7.7–8.0 (m, 2 H, 1-NH + 3-H), 8.0–8.35 (m, 2 H, 10-H + 6-H, J_{6-7} = 8.1 Hz), 8.48 (d, 1 H, 11-H, J_{11-10} = 9 Hz), 10.3 (br s, 1 H, OH), 12.71 (br s, 1 H, 5-NH)
26a	3 d	2M	66.5	198–200	$C_{23}H_{28}N_4 \cdot 2C_4H_4O_4 \cdot H_2O$	CHN	(D_2O) 0.95 (t, 2 × 3 H, (CH_3CH_2)$_2$), 1.5–1.8 (m, 5 H, β-CH_2 + 4-CH_3), 2.7–3.0 (m, 8 H, γ-CH_2 + α-CH_2 + (CH_2CH_3)$_2$), 5.71 (s, 4 H, CH = CH-maleate), 6.43 (br s, 1 H, 3-H), 6.9–7.2 (m, 4 H, 10-H + 11-H + 7-H + 8-H), 7.25–7.5 (m, 2 H, 6-H + 9-H)
26b	4 d	2M	67	150–160	$C_{24}H_{30}N_4O \cdot 2C_4H_4O_4 \cdot 1.5H_2O$	CHN	[(CD_3)$_2$SO] 1.02 (t, 6 H, (CH_3CH_2)$_2$), 1.7–2.0 (m, 2 H, β-CH_2), 3.5–3.8 (m, 2 H, α-CH_2), 3.95 (s, 3 H, OCH_3), 6.4–6.6 (m, 1 H, 1-NH), 7.34 (d × d, 1 H, 7-H, J_{7-6} = 9 Hz, J_{7-9} = 2.2 Hz), 7.52 (d, 1 H, 9-H), 7.66 (d, 1 H, 10-H, J_{10-11} = 8.8 Hz), 7.75 (s, 1 H, 3-H), 8.32 (d, 1 H, 11-H), 8.57 (br s, 1 H, 5-NH), 8.67 (d, 1 H, 6-H); NB: 4-CH_3 + γ-CH_2 + (CH_2CH_3)$_2$ signals are overlapped by (CD_3)$_2$SO
27a	5 d	2M	86	200–202	$C_{22}H_{26}N_4 \cdot 2C_4H_4O_4$	CHN	[(CD_3)$_2$SO] 1.9–2.3 (m, 2 H, β-CH_2), 2.80 (s, 3 H, 4-CH_3), 2.86 (s, 6 H, N(CH_3)$_2$), 3.1–3.3 (m, 2 H, γ-CH_2), 3.6–3.8 (m, 2 H, α-CH_2), 4.63 (s, 3 H, 5-NCH_3), 6.11 (s, 4 H, CH = CH-maleate), 7.4–7.8 (m, 4 H, NH + 3-H + 7-H + 8-H), 7.95 (d, 1 H, 10-H, J_{10-11} = 8.8 Hz), 8.1–8.3 (m, 1 H, 9-H), 8.54 (d, 1 H, 11-H), 8.7–8.8 (m, 1 H, 6-H)
27d	3 d[c]	2M	71	200–210	$C_{23}H_{28}N_4O \cdot 2C_4H_4O_4 \cdot H_2O$	CHN	($CDCl_3$) 1.8–2.1 (m, 2 H, β-CH_2), 2.40 (s, 6 H, N(CH_3)$_2$), 2.5–2.7 (m, 5 H, γ-CH_2 + 4-CH_3), 3.7–3.9 (m, 2 H, α-CH_2), 4.07 (s, 3 H, OCH_3), 4.43 (s, 3 H, 5-NCH_3), 6.91 (d, 1 H, 8-H, J_{8-7} = 7.6 Hz), 7.16 (br s, 1 H, NH), 7.4–7.6 (m, 1 H, 7-H), 7.84 (br s, 1 H, 3-H), 8.0–8.3 (m, 3 H, 6-H + 10-H + 11-H)
27e[e]	4.5 h	2M	58.5	195–210 dec	$C_{22}H_{26}N_4O \cdot 2C_4H_4O_4$	CHN	[(CD_3)$_2$SO] 1.9–2.2 (m, 2 H, β-CH_2), 2.79 (d, 3 H, 4-CH_3, J_{CH_3-3-H} = 0.8 Hz), 2.8–3.0 (m, 8 H, γ-CH_2 + N(CH_3)$_2$), 3.1–3.3 (m, 2 H, α-CH_2), 4.57 (s, 3 H, 5-NCH_3), 6.10 (s, 4 H, CH = CH-maleate), 7.08 (d, 1 H, 8-H, J_{8-7} = 7.6 Hz), 7.3–7.6 (m, 2 H, NH + 7-H), 7.76 (m, 1 H, 3-H), 8.0–8.3 (m, 2 H, 6-H + 10-H), 8.45 (d, 1 H, 11-H, J_{11-10} = 9 Hz), 10.3 (br s, 1 H, OH)
28a	2 d	2M	77	177–178	$C_{24}H_{30}N_4 \cdot 2C_4H_4O_4$	CHN[d]	(D_2O) 0.95 (t, 6 H, (CH_3CH_2)$_2$), 1.5–1.8 (m, 2 H, β-CH_2), 1.93 (d, 3 H, 4-CH_3, J_{CH_3-3-H} = 0.9 Hz), 2.7–3.1 (m, 8 H, (CH_2CH_3)$_2$ + γ-CH_2 + α-CH_2), 3.43 (s, 3 H, 5-NCH_3), 5.70 (s, 4 H, CH = CH-maleate), 6.46 (m, 1 H, 3-H), 6.8–7.2 (m, 4 H, 7-H + 8-H + 10-H + 11-H, J_{10-11} = 8.5 Hz), 7.2–7.7 (m, 2 H, 9-H, 6-H)
28b	42 h	FB	92	138–140	$C_{25}H_{32}N_4O$	CHN	[(CD_3)$_2$SO] 1.0 (t, 6 H, (CH_3CH_2)$_2$), 1.7–2.0 (m, 2 H, β-CH_2), 2.70 (s, 3 H, 4-CH_3), 3.5–3.8 (m, 2 H, α-CH_2), 3.96 (s, 3 H, OCH_3), 4.49 (s, 3 H, NCH_3), 6.6 (m, 1 H, NH), 7.30 (d × d, 1 H, 7-H, J_{7-6} = 9.2 Hz, J_{7-9} = 2.8 Hz), 7.61 (d, 1 H, 9-H), 7.65–7.8 (m, 2 H, 3-H + 10-H), 8.34 (d, 1 H, 11-H, J_{11-10} = 8.8 Hz), 8.65 (d, 1 H, 6-H); NB: γ-CH_2 + (CH_2CH_3)$_2$ signals are overlapped by (CD_3)$_2$SO
28c	2.5 h	2S	84	216–218	$C_{24}H_{30}N_4O \cdot 2CH_3SO_3H \cdot 3H_2O$	CHNS	(D_2O) 1.42 (t, 6 H, (CH_3CH_2)$_2$, 2.0–2.6 (m, 5 H, β-CH_2 + 4-CH_3), 2.91 (s, 6 H, ($CH_3SO_3^-$)$_2$, 3.2–3.7 (m, 8 H, γ-CH_2 + α-CH_2 + (CH_2CH_3)$_2$, 3.78 (s, 3 H, NCH_3), 6.8–7.3 (m, 4 H, 7-H + 9-H + 3-H + 10-H), 7.53 (d, 1 H, 11-H, J_{11-10} = 8.9 Hz), 7.75 (d, 1 H, 6-H, J_{6-7} = 10.2 Hz)
28d	2 d	2M	72	200–210	$C_{25}H_{32}N_4O \cdot 2C_4H_4O_4 \cdot H_2O$	CHN	[(CD_3)$_2$SO] 1.10 (t, 6 H, (CH_3CH_2)$_2$, 1.8–2.1 (m, 2 H, β-CH_2), 2.5–2.9 (m, 9 H, γ-CH_2 + 4-CH_3 + (CH_2CH_3)$_2$), 3.6–3.9 (m, 2 H, α-CH_2), 4.16 (s, 3 H, OCH_3), 4.60 (s, 3 H, NCH_3), 6.86 (br s, 1 H, NH), 7.21 (d, 1 H, 8-H, J_{8-7} = 7.6 Hz), 7.6–7.8 (m, 1 H, 7-H), 7.88 (br s, 1 H, 3-H), 8.0–8.5 (m, 3 H, 10-H + 6-H + 11-H, J_{10-11} = 9 Hz)
28e[e]	3 h	2M	54	200–215	$C_{24}H_{30}N_4O \cdot 2C_4H_4O_4 \cdot H_2O$	CHN	[(CD_3)$_2$SO] 1.22 (t, 6 H, (CH_3CH_2)$_2$, 2.0–2.2 (m, 2 H, β-CH_2), 2.77 (s, 3 H, 4-CH_3), 3.0–3.3 (m, 6 H, γ-CH_2 + (CH_2CH_3)$_2$), 3.6–3.9 (m, 2 H, α-CH_2), 4.58 (s, 3 H, NCH_3), 6.10 (s, 4 H, CH = CH-maleate), 7.07 (d, 1 H, 8-H, J_{8-7} = 7.9 Hz), 7.4–7.6 (m, 2 H, 7-H + NH), 7.74 (br s, 1 H, 3-H), 8.1–8.3 (m, 2 H, 10-H + 6-H), 8.46 (d, 1 H, 11-H, J_{11-10} = 9.6 Hz), 10.36 (br s, 1 H, OH)

[a] Rfx = heating times in days (d) or hours (h). [b] M = maleate; S = methanesulfonate; FB = free base. [c] Steel vessel at 180 °C. [d] Calcd: C, 63.36; H, 6.31; N,9.24. Found: C, 63.86; H, 6.22; N, 9.03. [e] Further purification was needed.

1-Chloro-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*]indoles 12a,b and 23a,b,d. General Procedure. A mixture of 4-methylpyrido[4,3-*b*]benzo[*e*]- or -benzo[*g*]indol-1-one (44 mmol), acetonitrile (100 mL), benzyltriethylammonium chloride (40 g, 176 mmol, 4 equiv), diethylaniline (27.9 mL, 176 mmol, 4 equiv), and phosphorus oxychloride (205 mL, 50 equiv) was heated under reflux for a 10-h period and evaporated under reduced pressure. Ice (200 g) was immediately added to the residue and the mixture was heated at reflux for 5 min and cooled. The resulting solid was filtered, washed with water, suspended in water, and treated with an excess of ammonia, and the precipitate was collected. It was recrystallized from ethanol or 2-ethoxy ethanol, giving the expected chloro derivatives as pale-yellow microcrystals (Table IX).

General Procedure for the Preparation of 1-Chloro-4,5-dimethyl-5*H*-pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*]indoles 13a,b and 24a,b,d. The starting compound (**12a,b** or **23a,b,d**, 15 mmol) was dissolved in dimethylformamide (100 mL) and treated, while stirring, with methyl iodide (1.15 mL, 18 mmol) in the presence of an excess of finely powdered potassium carbonate (16.6 g, 120 mmol), at ambient temperature for a 15-h period (TLC monitoring). The mixture was evaporated to dryness under reduced pressure, and the residue was taken up in water and extracted with methylene chloride. The dried (magnesium sulfate) solution was evaporated and the solid residue was recrystallized from ethanol to give yellow needles (Table X).

General Procedure for Obtaining 1-[[3-(Dialkylamino)propyl]amino]-4-methyl-5*H*-pyrido[4,3-*b*]benzo[*e*]- and -benzo[*g*]indoles 14–18 and 27–28 (a,b,d). The mixture of the required chloro derivative (10 mmol) and the [(dialkylamino)-propyl]amine (30 mL, large excess) was heated under N_2 and under reflux for the length of time indicated in Tables XI and XII. Excess diamine was evaporated under reduced pressure; the residue was taken up in diluted ammonia and extracted with methylene chloride. Evaporation of solvent provided a residue which was chromatographed on an alumina column with a 95/5 methylene chloride–ethanol mixture as eluent. Evaporation of the pure free base containing fractions of the expected compound gave a residue which was either recrystallized when solid or transformed into its dimaleate or dimethanesulfonate salt when oily, by treatment with an excess of maleic or methanesulfonic acid (3 equiv) in acetone as solvent (Tables XI and XII).

Preparation of Hydroxy Derivatives 14–18(c), 25, 27, 28(e), and 28c. General Procedure. The mixture of the methoxylated starting compound (1 g) and concentrated hydrobromic acid (*d* = 1.47, 25 mL) was stirred under argon and heated under reflux for the period indicated in Tables XI and XII. Evaporation to dryness under reduced pressure provided a solid residue, which was taken up in water and basified with an excess of ammonia. When solid, the precipitate was collected and air-dried. When oily, it was extracted with methylene chloride or ethyl acetate and then dried (magnesium sulfate) and evaporated. All compounds were then transformed into their salts (dimaleate or dimethanesulfonate) by treatment with 3 equiv of the acid, with acetone or methyl ethyl ketone as solvent. The salt was filtered, taken up in boiling acetone, collected, and air-dried, giving the expected compound. However, in the cases noted in Tables XI and XII, a further purification was necessary. The aqueous solution of the salified compound was treated with an excess of ammonia and extracted with methylene chloride. Evaporation of solvent provided a residue which was chromatographed on an alumina column, with a 95/5 methylene chloride–ethanol mixture as eluent. Evaporation of the pure compound-containing fractions then gave the pure free base, which was salified as mentioned above (Tables XI and XII).

In Vitro Antitumor Effects. P388 Cytotoxicity Determination. P388 cells in exponential growth were cultured in a RPMI 1640 medium supplemented with 2 mM glutamine, 10 μM 2-mercaptoethanol, and 10% (v/v) fetal calf serum, in a 5% CO_2 atmosphere at 37 °C. The drugs were dissolved in distilled water and added (0.04 mL) to the cells (10^5 cells/mL) on day 0. The cells were counted after a 96-h incubation with a ZBI Coulter counter and the results were expressed as the drug concentration (μg/mL) which inhibited 50% of the cell proliferation (ID_{50}). The ID_{50} values were estimated by regression analysis of the concentration/response data.

Multiple Tumor Disk Diffusion Soft Agar Assay. A hard bottom layer (0.8% noble agar) containing enriched media (1:1 CMRL–Fischer, horse serum 11%) was poured into 60-mm plastic dishes and allowed to solidify. A soft agar top layer (0.47% noble agar) containing enriched media (1:1 CMRL–Fischer, horse serum 11%) and titered cells (5×10^3 L1210; 10^6 solid tumors) was poured on top and allowed to solidify. The drug was added to a 6-mm Whatman filter-paper disk. The dried disk was then placed on the agar top layer. The plates were incubated 7–11 days and examined on an inverted microscope for measurement of the zone inhibition of the tumors. A difference of 250 or more units (1 unit = 30 μm) between the zone for the solid tumor and leukemia indicated a significant differential effect.[21,22]

In Vivo Antitumor Effects. The tumors were obtained from the National Cancer Institute Tumor Bank (NCI). Maintenance of the tumors and experimental antitumor activity evaluation were performed according to NCI general recommendations.[14]

P388 and L1210. P388 and L1210 leukemias were passaged in DBA/2 mice. For experimental purposes, viable P388 and L1210 leukemia cells (10^6 and 10^5, respectively) were inoculated ip on day 0 in B6D2 F1 mice under a volume of 25 mL/kg of body weight (at least five mice per group). Compounds were dissolved in distilled water and administered ip (25 mL/kg of body weight) at various doses, for four consecutive days (D_{1-4}). Vehicle and reference compounds **1** and **2** were administered under the same conditions. Animal mortality was checked daily and necropsy was performed on dead animals in order to differentiate tumor death (splenomegaly, ascite) from toxicity death. The antitumor activity (T/C) was evaluated according to the formula T/C × 100, where T = median day of survival of treated animals, C = median day of survival of control animals. Mice surviving for more than 50 days were considered cured. They were not included in the calculation of the median survival time. The following NCI criteria for activity were used:[14] P388 T/C > 150%, L1210 T/C > 120%.

MA 16C, B16, and C_{26}. These tumors were passaged in vivo in the inbred mouse strain of tumor origin: C3H (MA 16/C), C57BL/6 (B16), and BALB/c (C26) mice (10 animals per group). The animals were inoculated sc with a 1 mm^3 tumor fragment. Inbred strains of mice were used for the experiments except for C26, where CD_2F_1 mice were used. The drugs were administered iv on days 1–4, under a volume of 10 mL/kg. Animals were weighed daily and observed for toxicity. Animals were killed on day 14 for C26, day 15 for MA 16/C and B16, and day 17 for 3LL; the tumors were removed and weighed. The antitumor activity (T/C) was evaluated according to the formula T/C × 100 (T = median weight of tumors from treated animals, C = median weight of tumors from control animals). According to NCI criteria,[14] a compound is considered active when T/C × 100 is <42.

Lewis Lung Carcinoma. Viable Lewis lung carcinoma cells (10^6) were inoculated im into C57 BL/6 mice under a volume of 2.5 mL/kg (10 animals per group). The compounds were administered iv for four consecutive days, starting with day 1 (early 3 LL) or day 7 (advanced 3 LL). Animals were weighed daily. Survivors were killed on day 18, the lungs were removed and fixed in Fekete solution, and macroscopic lung metastasis were counted. Antitumor activity was evaluated according to the formula T/C × 100 (T = mean number of metastases in treated animals, C = mean number of metastasis in control animals).

Acknowledgment. A. Naudin and J. Pelette are gratefully acknowledged for technical assistance in biological studies. The secretarial assistance of L. Ramdani and N. Frey was greatly appreciated.

Registry No. **7a**, 530-93-8; **7b**, 2472-22-2; 8, 106689-40-1; **9a**, 125974-49-4; **9b**, 125974-44-9; **10a**, 125974-50-7; **10b**, 125974-45-0; **11a**, 125974-51-8; **11b**, 125974-46-1; **12a**, 125974-52-9; **12b**, 125974-47-2; **13a**, 125974-53-0; **13b**, 125974-48-3; **14a**, 125974-54-1; **14b**, 125974-68-7; **14c**, 125974-72-3; **15a**, 125974-55-2; **15b**,

(21) Corbett, T. H. *Proc. Ann. Meet. AACR* 1984, 25.
(22) Corbett, T. H.; Polin, L.; Wozniak, A. J.; Bissery, M. C.; Lo Russo, P. M.; Valeriote, F. A.; Baker, L. H. *Proc. Ann. Meet. AACR* 1988, *29*, 532.

125974-69-8; **15c**, 125974-73-4; **16b**, 125974-56-3; **16c**, 125974-74-5; **17a**, 125974-57-4; **17b**, 125974-70-1; **17c**, 125974-75-6; **18a**, 125974-58-5; **18b**, 125974-71-2; **18c**, 125974-76-7; **19a**, 530-93-8; **19b**, 1078-19-9; **19d**, 33892-75-0; **20a**, 125974-59-6; **20b**, 125974-77-8; **20d**, 125974-78-9; **21a**, 125974-60-9; **21b**, 125974-80-3; **21d**, 125974-79-0; **22a**, 125974-61-0; **22b**, 125974-82-5; **22d**, 125974-81-4; **23a**, 125974-62-1; **23b**, 125974-84-7; **23d**, 125974-83-6; **24a**, 125974-63-2; **24b**, 125974-86-9; **24d**, 125974-85-8; **25a**, 125974-64-3; **25d**, 125974-87-0; **25e**, 125974-88-1; **26a**, 125974-65-4; **26b**, 125974-89-2; **27a**, 125974-66-5; **27d**, 125974-90-5; **27e**, 125974-91-6; **28a**, 125974-67-6; **28b**, 125974-92-7; **28c**, 125974-93-8; **28d**, 125995-64-4; **28e**, 125974-94-9; 3-(dimethylamino)propylamine, 109-55-7; 3-(diethylamino)propylamine, 104-78-9; 3-(dimethylamino)-2-methylpropylamine, 6105-72-2.

Book Reviews

Synthesis and Applications of Isotopically Labelled Compounds 1988. Proceedings of the Third International Symposium. Innsbruck, Austria, 17–21 July, 1988. Edited by T. A. Baillie and J. R. Jones. Elsevier, New York. 1989. xxxvii + 823 pp. 17 × 24.5 cm. ISBN 0-444-87368-6.

This work chronicles the third meeting of its kind and the first at a European venue. Attended by slightly over 300 scientists from all over the world, the meeting was extraordinary in its setting and quality of participation covering a wide range of topics in isotope research.

Divided into seventeen sections, this volume reviews the contents of the symposium by the use of author-prepared abstracts which were directly reproduced. Among the topics discussed are radiation-induced reactions, new techniques in isotopic analysis, synthesis of isotopically labeled compounds via organometallic chemistry, and current trends and future prospects in the synthesis and applications of isotopically labeled compounds. One section is even devoted to the many posters presented at the meeting. As with the preceding volume, this book also contains a useful author and subject index and a valuable listing of the meeting participants with their affiliations and addresses.

With each succeeding symposium of this kind both the scientific merit of the meeting and published proceedings increase. The editors of this particular volume should be commended especially for the several years of diligent effort required to produce such a large volume of uniform quality contributions. This book will undoubtedly be a welcome addition to the libraries of those researchers working with isotopically labeled substances.

E. I. du Pont de Nemours & Co. **Crist N. Filer**
NEN Products
Boston, Massachusetts 02118

The Conformational Analysis of Cyclohexenes, Cyclohexadienes, and Related Hydroaromatic Compounds. Edited by Peter W. Rabideau. VCH Publishers, Inc., New York. 1989. 323 pp. 16 × 24 cm. ISBN 0-89574-702-7. $74.50.

This is a good book. It covers in detail the title subjects. While the conformational analysis of cyclohexane has been worked out exhaustively, there is no comparable review of cyclohexenes and the other derivatives discussed in this volume.

The book consists of eight chapters. The first and most detailed, by F. A. L. Anet, is on the conformational analysis of cyclohexene itself. The second, by J. B. Lambert, is concerned with exocyclic double bonds in six-membered carbocyclic rings. There are then chapters on both 1,3- and 1,4-cyclohexadienes and related compounds, on ^{13}C NMR, on applications to the structures at hand, and on force field calculations. There is a short chapter on heterocyclic partially unsaturated six-membered rings, but this is rather sketchy. Finally, there is a chapter on metabolites of carcinogenic hydrocarbons, as related to the above.

There is much useful information on the title compounds here, gathered together in one place, and well presented.

The University of Georgia **Norman L. Allinger**
Athens, Georgia 30602