Modulation of the Antitumor Activity by Methyl Substitutions in the Series of 7*H*-Pyridocarbazole Monomers and Dimers

P. Léon, C. Garbay-Jaureguiberry, M. C. Barsi, J. B. Le Pecq,† and B. P. Roques*

Département de Chimie Organique, U 266 INSERM, UA 498 CNRS, UER des Sciences Pharmaceutiques et Biologiques, 75006 Paris, France, and Unité de Physicochimie Macromoléculaire, Institut Gustave Roussy, 94805 Villejuif, France.
Received February 23, 1987

The structure of the dimeric antitumor drug ditercalinium (NSC 366241) [2,2′-([4,4′-bipiperidine]-1,1′-diyldi-2,1-ethanediyl)bis[10-methoxy-7*H*-pyrido[4,3-*c*]carbazolium] tetramethanesulfonate] was modified by introduction of methyl groups in various positions of the aromatic ring. Monomeric analogues with the nitrogen atom of the pyridinic ring in different positions have also been synthesized. Pharmacological properties and DNA interactions of the new compounds are reported. In contrast with the monomeric analogue of ditercalinium, which was inactive, methyl substitutions on the 10-methoxy-7*H*-pyrido[4,3-*c*]carbazolium in positions 6 or 7 led to monomers endowed with small but significant activity. As expected, dimerization of the methyl-substituted pyridocarbazoles yielded DNA bisintercalators with affinity slightly higher than that of the unsubstituted parent compounds. These dimers, characterized by a relatively better therapeutic index, have the same mechanism of action as ditercalinium. Otherwise, in monomeric and dimeric series, methyl substitution in position 4 or 5 provided inactive compounds unable to intercalate into DNA. All these results are in agreement with the previously proposed geometry for the complex of ditercalinium with DNA.

It is now widely agreed that DNA behaves as the target for a large number of antitumor agents. Among these, DNA intercalators (adriamycin, bleomycin, ellipticine, *m*-AMSA) represent an important class of clinically used drugs.[1-4] The antitumor properties of these molecules and their interaction with DNA or DNA regulatory proteins (i.e., polymerases) have been shown to be significantly correlated.[1-5] During the last few years, progress in the techniques of molecular biology allowed the demonstration that monointercalators such as *m*-AMSA, adriamycin, and ellipticine induce, at pharmacologically active doses, DNA strand breaks associated with topoisomerase II.[6-8]

However, whether or not these findings observed at the cellular level are relevant to the in vivo activity remains a crucial problem dependent on the pharmacokinetic and metabolic drug pathway.[9-11]

In the series of 7*H*-pyridocarbazoles structurally related to the 6*H*-pyridocarbazoles (ellipticines), dimeric bisintercalators endowed with antitumor properties have been prepared.[12] Among these, ditercalinium (NSC 366241), recently introduced in phase 1 clinical trials, elicits its antitumor activity through a new mechanism characterized by a delayed toxicity on L1210 cell cultures.[13]

Unlike *m*-AMSA, ditercalinium does not stimulate DNA cleavage by DNA topoisomerase II. In contrast, at low concentrations, ditercalinium prevents DNA relaxation and inhibits the relaxation induced by *m*-AMSA.[14] Ditercalinium has also been shown to be cytotoxic on *Escherichia coli pol A* mutants and not on *pol A uvr A* double mutants, suggesting that the dimer is able to induce in vivo DNA conformational changes recognized by the *uvr ABC* repair system in *E. coli*.[15] Moreover, some results suggest that, in mammalian cells, mitochondrial DNA could represent a specific target for this drug series.[16] The structure of the DNA bisintercalating complex of ditercalinium has recently been proposed from NMR studies of its binding to the self-complementary tetranucleotide $d(CpGpCpG)_2$.[17] Ditercalinium was shown to bisintercalate with the convex face of the pyridocarbazole oriented toward the sugar moiety and the linking chain lying in the major groove. Following on, from this model it was considered interesting to analyze the bisintercalating capacity of dimers whose convex face carried substituents of increased size and to determine their biological activity. Previously, enzymatic methylation of tRNAs[18] and RNA polymerase activity have been shown to be partially blocked by ellipticine derivatives[19] bearing methyl groups on positions 5 and 11. Thus, the presence of methyl groups on the ring of 7*H*-pyridocarbazole in addition to enhancement of DNA affinity through hydrophobic interactions could induce antitumor properties through inhibition of gene expression by interference with DNA or RNA methylation processes. Therefore, monomeric and dimeric series of 7*H*-pyrido-

† Institut Gustave Roussy.

(1) Waring, M. J. *Annu. Rev. Biochem.* **1981**, *50*, 159.
(2) Wilson, W. D.; Jones, R. L. *Adv. Pharmacol. Chemother.* **1981**, *18*, 17.
(3) Wakelin, L. *Med. Res. Rev.* **1986**, *6*, 275.
(4) Myers, C. E. In *Pharmacologic Principles of Cancer Treatment*; Chabner, B., Ed.; Saunders: Philadelphia, 1982; pp 416.
(5) Meienhofer, J.; Atherton, E. In *Structure–Activity Relationships among the Semisynthetic Antibiotics*; Perlman, D., Ed.; Academic: London, 1977; p 427.
(6) Rowe, T. C.; Wang, J. C.; Liu, L. F. *Mol. Cell. Biol.* **1986**, *6*, 985.
(7) Ross, W. E.; Bradley, M. C. *Biochim. Biophys. Acta* **1981**, *654*, 129.
(8) Potsemil, M.; Israel, M.; Silber, R. *Biochem. Pharmacol.* **1984**, *33*, 3137.
(9) Favaudon, V. *Biochimie* **1982**, *64*, 457.
(10) Schwartz, H. S. In *Molecular Aspects of Anticancer Drug Action*; Neidle, S., Waring, M. J., Eds.; Macmillan: London, 1983; p 93.
(11) Lown, J. W. In *Molecular Aspects of Anticancer Drug Action*; Neidle, S., Waring, M. J., Eds.; Macmillan: London, 1983; p 283.
(12) Roques, B. P.; Pélaprat, D.; Le Guen, I.; Porcher, G.; Goss, C.; Le Pecq, J. B. *Biochem. Pharmacol.* **1979**, *28*, 1811.
(13) Esnault, C.; Roques, B. P.; Jacquemin-Sablon, A.; Le Pecq, J. B. *Cancer Res.* **1984**, *44*, 4335.
(14) Markovits, J.; Pommier, Y.; Mattern, M. R.; Roques, B. P.; Le Pecq, J. B.; Kohn, K. W. *Cancer Res.* **1986**, *46*, 5821.
(15) Le Pecq, J. B.; Roques, B. P. In *Mechanisms of DNA Damage and Repair*; Simic, M. G., Grossman, L., Upton, A. C., Eds.; Plenum: New York, 1986; pp 219.
(16) Esnault, C.; Lambert, B.; Markovits, J.; Segal-Bendirdjian, E.; Muzard, G.; Garbay-Jaureguiberry, C.; Roques, B. P.; Le Pecq, J. B. In *Molecular Mechanisms of Carcinogenic and Antitumor Activity*; Chagas, C., Pullman, B., Eds.; Pontifica Academia Scientiarum: Vatican, in press.
(17) Delbarre, A.; Delepierre, M.; Garbay-Jaureguiberry, C.; Igolen, J.; Le Pecq, J. B.; Roques, B. P. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2155–2159.
(18) Jian, M. C.; Lin, G. W.; Knight, C. A.; Murlhert, R. B. *Cancer Res.* **1977**, *37*, 4202.
(19) Sethi, S. *Biochem. Pharmacol.* **1981**, *30*, 2026.

0022-2623/87/1830-2074$01.50/0 © 1987 American Chemical Society

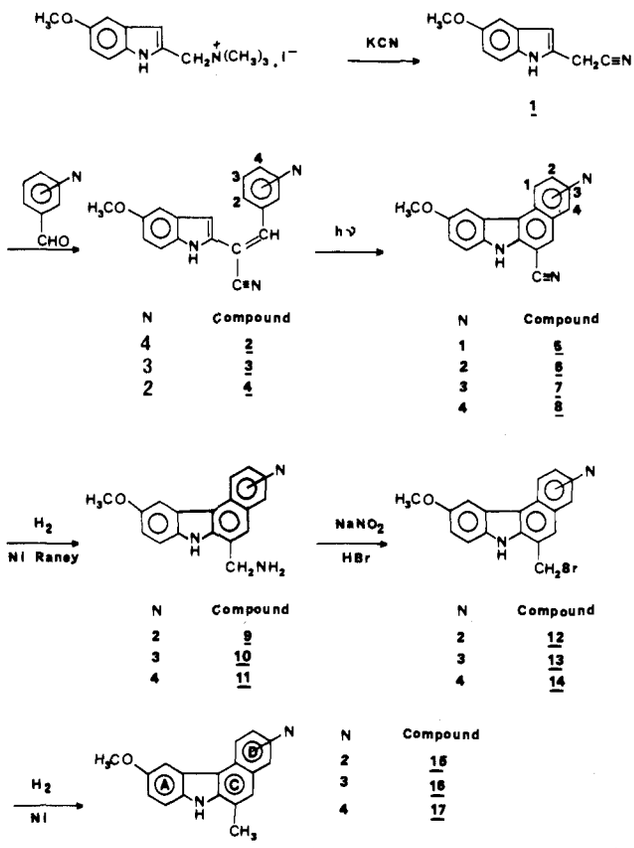


Figure 1. Synthesis of the different 10-methoxy-7*H*-pyridocarbazole rings methylated in position 6.

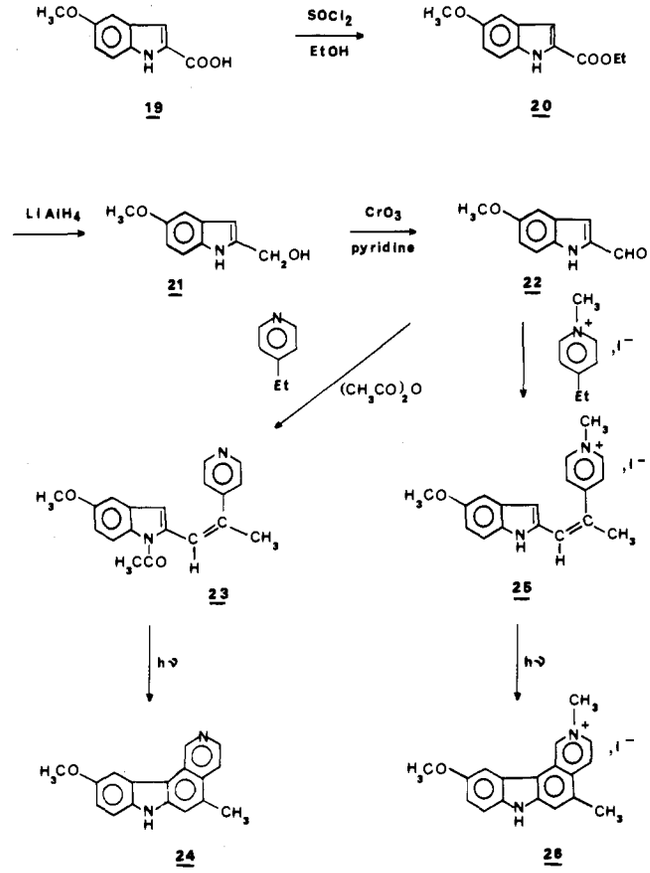


Figure 2. Synthesis of 10-methoxy-5-methyl-7*H*-pyrido[4,3-*c*]-carbazole (**24**) and its methiodide salt (**26**).

[4,3-*c*]carbazoles (N_2 series) and 7*H*-pyrido[3,4-*c*]carbazoles (N_3 series) substituted by methyl groups on their convex face were prepared and tested for antitumor activity.

Chemistry

Pyridocarbazoles methylated in position 5, 6, or 7 were obtained by different chemical pathways. Methylation of the 7*H*-pyridocarbazoles on position 7 was performed by reaction with NaH at room temperature followed by addition of methyl iodide.[20] Methylation at the 5- or 6-position required a total synthesis of the pyridocarbazole ring (Figures 1 and 2). Among the different methods (see ref 21) reported for the synthesis of the 7*H*-pyridocarbazole ring, the photochemical cyclization pathway[21-23] was chosen because all the different pyridocarbazole isomers could be obtained from the appropriately substituted indolylpyridylethylene. The precursors were prepared by two different methods according to the final position (5 or 6) of the methyl group.

The 6-methyl-7*H*-pyridocarbazoles were obtained in three steps from the 6-cyano derivatives. These derivatives were synthesized by condensation of the 5-methoxyindole-2-acetonitrile with differently substituted formylpyridines providing the corresponding 2-(α-indolyl)-3-pyridylacrylonitriles (Figure 1) and followed by subsequent photocyclization. As already observed by Husson et al.,[23] the condensation carried out with 3-formylpyridine afforded the 2-(5-methoxyindol-2-yl)-3-(3-pyridyl)acrylonitrile (**3**), which led by the same photoirradiation step in oxidative conditions to a mixture of 7*H*-pyrido[3,4-*c*]carbazole (**7**) (nitrogen in position 3) and 7*H*-pyrido[3,2-*c*]-carbazole (**5**) (nitrogen in position 1) in the ratio N_1/N_3 = 4/5 (Table I).

Table I. Condensation of 5-Methoxyindole-2-acetonitrile (1) with Differently Substituted Formylpyridine followed by Photochemical Cyclization

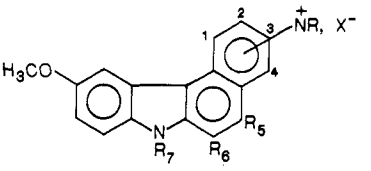
formyl-pyridine	photo-irradn time, h	N position on 7*H*-pyrido-carbazole	% yield	compd
para	30	2	75	**6**
meta	10	3	45	**7**
		1	35	**5**
ortho	50	4	31	**8**

In each series (nitrogen on the different positions), the nitrile group was transformed into a methyl group by reduction under pressure and diazotization of the intermediate amine in concentrated HBr, leading to a bromomethyl group, which was subsequently hydrogenated by using Raney Ni as a catalytic agent. The dimethylated compound **18** was obtained by methylation of the pyrrolic nitrogen of compound **15** as described above.

10-Methoxy-5-methyl-7*H*-pyrido[4,3-*c*]carbazole (**24**) was synthesized through condensation of 5-methoxyindole-2-carboxaldehyde (**22**) and 4-ethylpyridine in acetic anhydride yielding 1-(1-acetyl-5-methoxyindol-2-yl)-2-(4-pyridyl)propene (**23**). This method is derived from that described by Cohylakis et al.[24] in the synthesis of the larger alkaloid subincanine. As observed by these authors, both ring closure and photocatalyzed hydrolysis of the *N*-acetyl

(20) Dalton, L. K.; Demerac, S.; Elmes, B. C.; Loder, J. W.; Swan, J. M.; Tertei, T. *Aust. J. Chem.* **1967**, *20*, 2745.
(21) Pélaprat, D.; Oberlin, R.; Le Guen, I.; Roques, B. P.; Le Pecq, J. B. *J. Med. Chem.* **1980**, *23*, 1330.
(22) De Silva, O.; Snieckus, V. *Synthesis* **1971**, *5*, 254.
(23) Husson, H. P.; Thal, C.; Potier, P.; Wenkert, E. *J. Org. Chem.* **1970**, *35*, 442.
(24) Cohylakis, D.; Hignett, G. J.; Lickman, K. V.; Joule, J. A. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1518.

Table II. 7*H*-Pyridocarbazole Monomers: Intercalation, DNA Affinity, and Antitumor Activity



no.	N-R	X^-	R_5	R_6	R_7	ΔL[a] (pH 7.4)	$K \times 10^5$ M^{-1} (pH 7.4)	cellular toxicity on L1210: growth inhibn: ED_{50},[b] μM	cellular toxicity on L1210: cloning efficiency: CE_{37},[c] μM	antitumor act.[d]: MTD,[e] mg/kg	antitumor act.[d]: OD,[f] mg/kg	antitumor act.[d]: T/C %[g]
38	N_2-CH_3	I^-	H	H	H	2.3	2.9	0.95	0.77	50	25	NS[h]
26	N_2-CH_3	I^-	CH_3	H	H	0	0.6	>2.5	3.71	NT[i]	NT	NT
27	N_2-CH_3	I^-	H	CH_3	H	2.7	1.8	0.06	0.03	50	25	125
39	N_2-CH_3	I^-	H	H	CH_3	2.3	7.0	0.22	0.74	25	17	122
30	N_2-CH_3	I^-	H	CH_3	CH_3	1.83	10	0.22	0.60	NT	NT	NT
40	N_3-CH_3	I^-	H	H	H	3.04	2	>2.5	NT	50	25	NS
28	N_3-CH_3	I^-	H	CH_3	H	2.37	3	0.30	0.15	NT	NT	NT
41	N_3-CH_3	I^-	H	H	CH_3	2.90	3.1	2.22	NT	150	75	115
42	N_4-CH_3	I^-	H	H	H	1.8	0.4	19.2	>2.5	NT	NT	NT
29	N_4-CH_3	I^-	H	CH_3	H	0	0.61	>2.5	>2.5	NT	NT	NT
43	N_2-$(CH_2)_2$-c-$N(CH_2)_5$	Cl^-	H	H	H	2.19	5.4	0.25	0.14	10	5	NS
31	N_2-$(CH_2)_2$-c-$N(CH_2)_5$	Cl^-	CH_3	H	H	1.04	1.0	2.24	NT	NT	NT	NT
32	N_2-$(CH_2)_2$-c-$N(CH_2)_5$	Cl^-	H	CH_3	H	1.91	9.3	0.11	0.10	23	5	123
44	N_2-$(CH_2)_2$-c-$N(CH_2)_5$	Cl^-	H	H	CH_3	2.33	12	0.67	3.36	10	5	NS
33	N_2-$(CH_2)_2$-c-$N(CH_2)_5$	Cl^-	H	CH_3	CH_3	2.79	6.1	0.44	NT	10	10	NS

[a] ΔL: slope of the plot for sonicated calf thymus DNA lengthening as log $[\eta]/[\eta_0]$ vs. log $(1 + 2r)$ (see Experimental Section). [b] Dose (μM) that inhibits 50% of the cell growth after 24-h exposure to the drug. [c] Dose (μM) required to inhibit the cloning efficiency to a factor of 37%. [d] Antitumor activity in vivo on L1210-infected mice. [e] MTD: maximal tolerated dose (mg/kg). [f] OD: optimal dose (mg/kg). [g] T/C %: treated mean survival time per control mean survival time. [h] NS: nonsignificant values. [i] NT: not tested.

group took place during the photocyclization of **23** into **24**. Its methiodide derivative **26** was obtained in a rather similar way: condensation of **22** with 4-ethylpyridinium methiodide in piperidine following the method described by Jerchel et al.[25] and the subsequent photocyclization provided **26** (Figure 2).

The intermediate 5-methoxyindole-2-carboxaldehyde (**22**) was obtained from 5-methoxyindole-2-carboxylic acid (**19**) after esterification, reduction by $LiAlH_4$, and oxidation with CrO_3–pyridine (Figure 2).

Quaternizations performed as previously described[21] with either methyl iodide or *N*-(2-chloroethyl)piperidine afforded the monomers, while the dimers were obtained by reaction with the bifunctional 1,1′-bis(2-chloroethyl)-4,4′-bipiperidine as reported in ref 26. Attempts to quaternize the nitrogen N_1 of the 7*H*-pyrido[3,2-*c*]carbazoles were unsuccessful as already observed in similarly constrained heterocycles.[21,23]

Interaction with DNA:DNA Affinity and Viscometric Studies

DNA binding affinities were determined by using a fluorimetric assay based upon the competition of the pyridocarbazole monomers or dimers with ethidium bromide or ethidium dimer, respectively.[27]

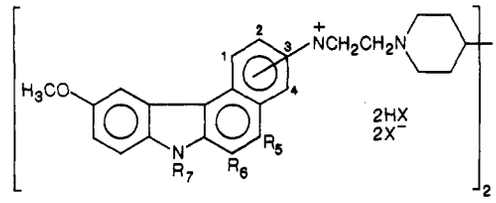
The DNA intercalating ability of the 7*H*-pyridocarbazoles was determined by viscometry, measuring the lengthening of sonicated calf thymus DNA in the presence of various amounts of drugs.[28]

Monomers. As shown in Table II, the DNA affinity of the various 7*H*-pyridocarbazole monomers lies in the 10^4–10^6 M^{-1} range as observed for nonmethylated ones.[21] Nevertheless, substitution of the 7*H*-pyridocarbazole ring by a methyl group strongly modulates these affinity constants. Indeed, methyl substitution in position 7 (compounds **39**, **41**, **44**) and introduction of two methyl groups on positions 6 and 7 (compounds **30** and **33**) increase the DNA affinity in each series. Methylation on position 6 leads to compounds (**27**, **28**, **29**, **32**) with affinity constants in the same range as that for the corresponding unsubstituted monomers. In contrast, methylation on position 5 produces a drastic decrease in DNA affinity (compounds **26** and **31**). In the N_4 series, low DNA affinity constants are observed (compounds **29** and **42**). Moreover, it is noticeable that the modulation in DNA affinity, brought about by the methyl groups in different positions, can be roughly correlated with the intercalating ability of the substituted monomers. Thus the 5-methylated compounds (**26** and **31**), which do not intercalate into DNA, as clearly shown by their inability or very weak capacity to lengthen sonicated DNA, exhibit a significantly lower DNA affinity. The nonintercalating ability of these compounds is probably due to steric hindrance between the 5-methyl group and the nucleotide sugar backbone. The geometry that we recently proposed for the complex between the self-complementary nucleotide $d(CpGpCpG)_2$ and compound **43**, the monomeric analogue of ditercalinium, would support this hypothesis.[29]

Similarly, the methyl quaternization in position 4 (compound **42**) should also provoke steric hindrance with the sugar moiety. However, the capability of this compound to intercalate into DNA (Table II) is certainly related to a slight change in the complex geometry locating

(25) Jerchel, D.; Heck, H. E. *Justus Liebigs Ann. Chem.* **1958**, *613*, 171.
(26) Pélaprat, D.; Delbarre, A.; Le Guen, I.; Roques, B. P.; Le Pecq, J. B. *J. Med. Chem.* **1980**, *23*, 1336.
(27) Gaugain, B.; Barbet, J.; Capelle, N.; Roques, B. P.; Le Pecq, J. B. *Biochemistry* **1978**, *17*, 5078.
(28) Saucier, J. M.; Festy, B.; Le Pecq, J. B. *Biochimie* **1971**, *53*, 973.
(29) Delepierre, M.; Delbarre, A.; Langlois d'Estaintot, B.; Igolen, J.; Roques, B. P. *Biopolymers* **1987**, *26*, 981.

Table III. 7*H*-Pyridocarbazole Dimers: Intercalation, DNA Affinity, and Antitumor Activity



								cellular toxicity on L1210				
										antitumor act.		
no.	X^-	N	R_5	R_6	R_7	ΔL (pH 7.4)	$K \times 10^7$ M^{-1} (pH 7.4)	growth inhibn: ED_{50}, μM	cloning efficiency: CE_{37}, μM	MTD, mg/kg	OD, mg/kg	T/C %
45[b]	$CH_3SO_3^-$	2	H	H	H	3.6	1	0.19	0.024	10	10	182
34	Cl^-	2	CH_3	H	H	0.	0.5	>1	0.42	6	2	120
35	Cl^-	2	H	CH_3	H	6.16	50	0.37	0.01	11	7	172
46	Cl^-	2	H	H	CH_3	3.6	2	0.36	0.006	10	2.5	178
36	Cl^-	2	H	CH_3	CH_3	4.00	10	3.27	10.9	NT	NT	NT
47	Cl^-	3	H	H	H	1.3	0.3			100	20	130
37	Cl^-	3	H	CH_3	H	0.56	1	>1	0.60	NT	NT	NT
48	Cl^-	3	H	H	CH_3	0.56	0.6		3.93	15	7.5	NS

[a] See Table II footnotes *a–i*. [b] Ditercalinium.

the N_4-CH_3 out of the major groove. In contrast, disubstitution on N_4 and C_6 certainly affords additional steric constraints preventing intercalation of compound **29**.

Regarding the geometry of the proposed model,[29] the substitution on position 6 or 7 or even disubstitution on 6 and 7 in both the N_2 and N_3 series does not induce any steric hindrance with the sugar backbone, in accordance with the DNA intercalating ability of the corresponding quaternized monomers (**27**, **28**, **32**, **39**, **41**, **44**).

Dimers. Since active monomers were found in the methylated series bearing nitrogen at position 2 (N_2) or 3 (N_3), only the dimers belonging to these series were synthesized. In the dimeric series, the modulating effect of the methyl substitution on the DNA binding properties is amplified as compared to monomers. Whereas the monomers belonging to the 7*H*-pyrido[3,4-*c*]carbazole series (N_3) intercalate into DNA, none of the corresponding dimers was shown to bisintercalate, and accordingly these compounds (**37**, **47**, **48**) elicited low DNA affinities (Table III). In contrast, bisintercalating dimers were obtained in the 7*H*-pyrido[4,3-*c*]carbazole series (N_2), methylated or not.[26] In this series, the effect of the methyl groups on DNA binding properties is rather different from that observed in the corresponding monomeric series. Thus, while methylation at position 7 does not produce a significant increase in the DNA affinity of compound **46**, methylation at position 6 and dimethylation at positions 6 and 7 lead to bisintercalators endowed with the highest DNA affinity (compounds **35**, **36**) in the series. This might be explained by additional van der Waals interactions between the methyl groups and DNA, as shown in the complex between ditercalinium and $d(CpGpCpG)_2$. As expected, methylation at position 5 provides a dimer **35** characterized by a low DNA affinity (5×10^6 M^{-1}) in accordance with its inability to intercalate (Table III). As in the case of the corresponding monomer **26**, these results are probably related to steric hindrance between the 5-methyl substituent and the sugar moiety avoiding DNA intercalation, following the model established from NMR analyses in the case of ditercalinium.[17]

Antitumor Properties

The antitumor activities of the 7*H*-pyridocarbazoles were evaluated by using the L1210 murine leukemia because of the good predicting value of this tumor in human cancer chemotherapy.[30] In addition, the cytotoxicity of the compounds was also determined on L1210 cells in vitro, by measurements of both growth inhibition and cloning efficiency. The results are presented in Tables II and III.

Monomers. As normally observed, a direct relationship between in vitro cytotoxic effects and in vivo antitumor activity was found, as illustrated for an active compound such as **27** and inactive compounds such as **40** and **41** (Table II). Therefore, cytotoxic activities on L1210 cells were measured for all the monomers, but only the more toxic molecules were retained for investigation of their antitumor potency. Some monomeric drugs exhibit weak but significant antitumor activity with T/C values in the order of 125. These compounds belong to the N_2 series, except for the less active monomer **41**, which belongs to the N_3 series. They are characterized by the presence of a methyl group on either position 6 or 7 (compounds **27**, **32**, **39**, **41**). In contrast, nonmethylated monomers[31] (**38**, **40**, **43**) and the dimethylated compound **33** are devoid of antitumor properties although they intercalate into DNA with affinities not significantly different from those of active compounds. The presence of a methyl group on position 6 or 7 in the N_2 series seems to induce some antitumor potency. This suggests the possible involvement in the cytotoxic mechanism of an enzymatic system that might interfere selectively with the drug–DNA complex at the level of the methyl group. As expected, 5-methyl-substituted derivatives **26** and **31** are devoid of biological activity in accordance with their nonintercalative mode of interaction and their associated low DNA affinity. Similarly, compounds **29** and **42** belonging to the N_4 series have no intercalating properties and are devoid of antitumor activity. Thus, intercalating ability, high DNA affinity, and methyl substitution at position 6 or 7 appear as essential parameters to obtain compounds endowed with antitumor activity. However, no direct correlation can be drawn between these different parameters, since several compounds (i.e., compound **44** methylated on top 7) are devoid of antitumor potency, although they display high DNA affinity.

(30) Zubrod, C. G. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 1042.
(31) Garbay-Jaureguiberry, C.; Laugâa, P.; Delepierre, M.; Laalami, S.; Muzard, G.; Le Pecq, J. B.; Roques, B. P. *Anti-Cancer Drug Des.* 1987, *1*, 323.

Dimers. Results of antitumor tests presented in Table III show a different behavior for the derivatives belonging to the N_2 and N_3 series. Highly active compounds are found in the N_2 series (compounds **35**, **45**, **46**) whereas dimers belonging to the N_3 series do not display significant antitumor potency (compounds **37**, **47**, **48**). There appears to be a correlation between bisintercalating ability and antitumor properties, since dimers of the N_3 series do not bisintercalate into DNA. Moreover, in the N_2 series, compound **34**, which seems to be unable to intercalate, is inactive. This is in agreement with the results observed in the monomeric series, where methylation on position 5 leads to nonintercalating compounds devoid of antitumor properties. However, the 6,7-dimethylated compound **36**, although bisintercalating and endowed with high DNA affinity, is completely devoid of cytotoxicity. This result emphasizes the importance for antitumor potency of the geometry of the DNA complex, which might be strongly modified by the presence of two contiguous methyl groups.

In the N_2 series, no direct correlation is observed between DNA affinity and L1210 cytotoxicity or antitumor properties. On L1210 cells, a large difference is observed between the growth rate inhibition (ED_{50}) and the cloning efficiency (CE_{37}) for the 6- and 7-methyl-substituted compounds. This was previously reported in the case of ditercalinium and related compounds[31] and attributed to their delayed toxicity.[13] Recent studies[13-16] show that ditercalinium (**45**) and its 7-methyl-substituted analogue **46** display the same mechanism of action, which could also be shared by the 6-methylated compound **35**. Although the increases in life span (reported as T/C % value) are not very different for ditercalinium **45** and its 6- or 7-methylated analogues **35** and **46**, these latter compounds display better therapeutic indexes as shown by their smaller optimal active doses (Table III).

Conclusion

Methyl substitution on well-defined positions (6 or 7) of inactive 7*H*-pyrido[4,3-*c*]carbazolium monomers gives rise to derivatives endowed with small but significant antitumor activity. Moreover, in the 7*H*-pyridocarbazole series the location of the substitution is crucial, as shown by the activity of compounds methylated on C_6 or N_7 while derivatives bearing a methyl group on positions 4 or 5 are inactive and nonintercalating.

Concerning the dimers, it appears clearly that the size and the steric requirements for the intercalating sites are crucial factors for possible insertion into DNA of tetracyclic compounds as large as 7*H*-pyridocarbazoles. Thus, in the 7*H*-pyrido[3,4-*c*]carbazole series (N_3 series), methyl substitution does not produce antitumor agents in accordance with the inability of the dimers to intercalate. In the 7*H*-pyrido[4,3-*c*]carbazole series (N_2 series), the bisintercalating ability of the dimers bearing a methyl group in position 6 or 7 seems to indicate that the geometry of their DNA complexes is not greatly different from that found for the complex of ditercalinium with $d(CpGpCpG)_2$ since, in the proposed geometry, the positions 6 or 7 remain easily accessible. According to these assumptions, the replacement of a 6-methyl by an ethyl group leads to a new ditercalinium analogue endowed with bisintercalative and antitumor properties.[32]

The presence of these additional methyl groups induces only a slight increase in the antitumor potency, but the derivatives seem to be active at lower therapeutic doses.

The results suggest that the antitumor activity of the monomers might originate from a mechanism of action quite different from that of the dimeric ditercalinium. It could resemble, in some aspects, that occurring in the 5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole (ellipticine), in which the 5-methyl substituent was shown to be absolutely essential for activity.[33] Accordingly in the 7*H*-pyridocarbazole series, the 6- or 7-methyl groups could play the same role as the 5-methyl group in ellipticine.

Thus the location of the methyl substitution appears to be a crucial parameter for the DNA complex formation and antitumor activity as already observed in different series such as actinomycins,[34] ellipticines,[33] and bisquinaldines.[35]

Experimental Section

Melting points were determined on a Kofler apparatus and were not corrected. The structures of all products were established by ^1H NMR spectra obtained on a Bruker 270-MHz spectrometer. Mass spectra were obtained on a RIBERMAG R.10.10.C spectrometer. Where analyses are indicated only by the symbols of elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

5-Methoxyindole-2-acetonitrile (1). 2-[(Dimethylamino)-methyl]-5-methoxyindole methiodide (17.17 g, 51.2 mmol) was dissolved in 250 mL of anhydrous methanol under nitrogen. KCN (11.5 g, 3.5 × 51.2 mmol) dissolved in 250 mL of anhydrous methanol was added, and the mixture was refluxed for 18 h with stirring. The solution was then concentrated in vacuo to 100 mL, poured into water, and extracted with ether (3 × 100 mL). The extracts were washed with water (2 × 100 mL), dried over sodium sulfate, filtered, and concentrated in vacuo. The nitrile 1 crystallized: yield 6.36 g (67%); mp >290 °C. Anal. ($C_{11}H_{10}N_2O$) C, H, N.

2-(5-Methoxyindol-2-yl)-3-(4-pyridyl)acrylonitrile (2). To a stirred solution of 3.61 g (19.41 mmol) of the nitrile 1 in 125 mL of methanol were added simultaneously in 10 min at room temperature a solution of sodium methoxide [600 mg of sodium (1.1 × 19.41 mmol) in 50 mL of methanol] and a solution of 2.1 mL (1.1 × 19.41 mmol) of 4-formylpyridine in 30 mL of methanol. Stirring was maintained for 1 h at 20 °C, and the crystalline suspension was filtered to give 3.24 g (yield 61%) of the desired nitrile **2**. The filtrate was concentrated in vacuo, and benzene was added to the residue; 0.26 g of **2** crystallized, **2**: total yield 3.50 g (66%); mp 240 °C. Anal. ($C_{17}H_{13}N_3O$) C, H, N.

2-(5-Methoxyindol-2-yl)-3-(3-pyridyl)acrylonitrile (3) **and 2-(5-Methoxyindol-2-yl)-3-(2-pyridyl)acrylonitrile** (4). Compounds **3** and **4** were similarly prepared from 3- and 2-formylpyridine, respectively. **3**: yield 70%; mp 210 °C. Anal. ($C_{17}H_{13}N_3O$) C, H, N. **4**: yield 51%; mp 170 °C. Anal. ($C_{17}H_{13}N_3O$) C, H, N.

6-Cyano-10-methoxy-7*H*-pyrido[4,3-*c*]carbazole (6). A solution of 1.7 g (6.18 mmol) of the nitrile **2** with 0.3 g (1.18 mmol) of iodine in 1500 mL of 95% ethanol was irradiated for 30 h (Hanau TQ 150 photoreactor). The nitrile **6** crystallized during the reaction. It was filtered and recrystallized from DMF: yield 1.27 g (75%); mp >290 °C. Anal. ($C_{17}H_{11}N_3O$) C, H, N.

6-Cyano-10-methoxy-7*H*-pyrido[3,4-*c*]carbazole (7) **and 6-Cyano-10-methoxy-7*H*-pyrido[3,2-*c*]carbazole** (5). Compounds **7** and **5** were similarly obtained from **3** (after 10-h irradiation). In this case a final chromatography over a silica gel column ($CHCl_3$–Et_2O, 2:1) allowed separation of the two pyridocarbazoles **7** and **5**. **7**: yield 46%; mp >290 °C. Anal. ($C_{17}H_{11}N_3O$) C, H, N. **5**: yield 31%; mp >290 °C. Anal. ($C_{17}H_{11}N_3O$) C, H, N.

6-Cyano-10-methoxy-7*H*-pyrido[2,3-*c*]carbazole (8). Compound **8** was similarly synthesized (time of irradiation, 49

(32) Léon, P.; Garbay-Jaureguiberry, C.; Le Pecq, J. B.; Roques, B. P. *Tetrahedron Lett.* **1985**, *26*, 4929.

(33) Hayat, M.; Mathé, G.; Janot, M. M.; Potier, P.; Dat-Xuong, N.; Cavé, A.; Sevenet, T.; Kan-Fan, C.; Poisson, J.; Miet, J.; Le Men, J.; Le Goffic, F.; Gouyette, A.; Ahond, A.; Dalton, L. K.; Connors, T. A. *Biomed. Express* **1974**, *21*, 101.

(34) Muller, W.; Crothers, D. M. *J. Mol. Biol.* **1968**, *35*, 251.

(35) Sinha, B. K.; Philen, R. M.; Sato, R.; Cysyk, R. L. *J. Med. Chem.* **1977**, *20*, 1528.

h). Chromatography over a silica gel column ($CHCl_3$–MeOH, 20:1) gave the pure pyridocarbazole **8**: yield 31%; mp 208 °C. Anal. ($C_{17}H_{11}N_3O$) C, H, N.

6-(Aminomethyl)-10-methoxy-7*H*-pyridocarbazoles 9–11. One gram (7.4 mmol) of the nitrile **6**, **7**, or **8** was dissolved in 100 mL of HMPT saturated with ammonia. One gram of Raney nickel was added and the pyridocarbazole hydrogenated under pressure (10 bars) for 3.5 h. The mixture was filtered and concentrated in vacuo. H_2O (30 mL) was added to the residue, and the precipitate was filtered and dissolved in 100 mL of aqueous 0.2 N HCl.

After extraction with $CHCl_3$ (5 × 100 mL), the aqueous phase was basified to pH 12 with 2 N sodium hydroxide and the 6-(aminomethyl)pyridocarbazole precipitated. It was filtered, washed with water, and dried in vacuo. **6** yielded **9**: 0.97 g (95%): mp 260 °C. Anal. ($C_{17}H_{15}N_3O$) C, H, N. **7** yielded **10**: 0.58 g (57%): mp >290 °C. Anal. ($C_{17}H_{15}N_3O$) C, H, N. **8** yielded **11**: 0.61 g (60%): mp >290 °C. Anal. ($C_{17}H_{15}N_3O$) C, H, N.

6-(Bromomethyl)-10-methoxy-7*H*-pyridocarbazolium Hydrobromides 12–14. A solution of 0.2 g (1.6 × 1.8 mmol) of sodium nitrite in 5 mL of H_2O was added in 30 min to a stirred solution of 0.5 g (1.8 mmol) of the amine **9–11** in 13 mL of H_2O and 1 mL of 48% aqueous HBr. Stirring was continued for 30 min at room temperature, and the mixture was filtered. The red solid thus obtained was dried in vacuo. **9** yielded **12**: 0.62 g (82%): mp >290 °C. Anal. ($C_{17}H_{13}BrN_2O$) C, H, N, Br. **10** yielded **13**: 0.63 g (83%): mp >290 °C. Anal. ($C_{17}H_{13}BrN_2O$) C, H, N, Br. **11** yielded **14**: 0.45 g (59%): mp >290 °C. Anal. ($C_{17}H_{13}BrN_2O$) C, H, N, Br.

10-Methoxy-6-methyl-7*H*-pyridocarbazoles 15–17. The hydrobromide **12–14** (0.62 g, 1.64 mmol) was dissolved in 125 mL of methanol. Raney nickel (3.5 g) was added, and the suspension was hydrogenated under atmospheric pressure for 4 h. The mixture was filtered and evaporated in vacuo. The resulting brown solid was stirred for 30 min in a mixture of $CHCl_3$–MeOH–1 N NaOH (10:40:5). After filtration, the solution was concentrated in vacuo to 25 mL and allowed to stand at room temperature overnight. A brown solid was isolated and chromatographed over a silica gel column ($CHCl_3$–MeOH, 9:1). **12** yielded **15**: 0.20 g (52%): mp 268 °C. Anal. ($C_{17}H_{14}N_2O$) C, H, N. **13** yielded **16**: 0.06 g (20%): mp 248 °C. Anal. ($C_{17}H_{14}N_2O$) C, H, N. **14** yielded **17**: 0.13 g (35%): mp 215 °C. Anal. ($C_{17}H_{14}N_2O$) C, H, N.

10-Methoxy-6,7-dimethyl-7*H*-pyrido[4,3-*c*]carbazole (18). Sodium hydride (0.02 g, 1.9 × 0.38 mmol) was added under nitrogen to a stirred solution of 0.10 g (0.38 mmol) of the pyridocarbazole **15** in 2 mL of anhydrous DMF. The solution was stirred for 10 min, and 0.05 mL (2.1 × 0.38 mmol) of methyl iodide diluted in 0.1 mL of anhydrous DMF was added over 1 h. The mixture was stirred at room temperature for 60 h in darkness, and the solvent was evaporated in vacuo. Compound **18** recrystallized from acetone–ethyl acetate (1:1): yield 0.076 g (72%); mp 210 °C. Anal. ($C_{18}H_{16}N_2O$) C, H, N.

Ethyl 5-Methoxyindole-2-carboxylate (20). To a stirred suspension of 5.05 g (26.4 mmol) of 5-methoxyindole-2-carboxylic acid (**19**) in 25 mL of anhydrous ethanol was added at −5 °C in 20 min 3.7 mL (1.8 × 26.4 mmol) of pure thionyl chloride. The mixture was allowed to warm to room temperature and then refluxed for 4 h. The resulting solid **20** was filtered, washed with *n*-hexane, and thoroughly dried in vacuo: yield 5.12 g (89%); mp 157 °C. Anal. ($C_{12}H_{13}NO_3$) C, H, N.

2-(Hydroxymethyl)-5-methoxyindole (21). Ester **20** (5.02 g, 22.8 mmol) was dissolved in 65 mL of Et_2O–THF (1:1). This solution was added dropwise over 1 h to a suspension of 1.25 g (1.45 × 22.8 mmol) of $LiAlH_4$ in 48 mL of anhydrous ether. The temperature reached 35 °C, and the mixture was refluxed for 1.5 h. H_2O (80 mL) was added. The organic extracts were washed with H_2O (100 mL), dried (Na_2SO_4), and evaporated in vacuo to give **21**: yield 3.94 g (98%); mp 83 °C. Anal. ($C_{10}H_{11}NO_2$) C, H, N.

5-Methoxyindole-2-carboxaldehyde (22). Compound **21** (2 g, 11.3 mmol) was dissolved in 6 mL of anhydrous pyridine. This solution was added over 40 min to a solution of 6.8 g (6 × 11.3 mmol) of CrO_3 in 170 mL of CH_2Cl_2 and 10.7 mL of pyridine. The mixture was stirred at room temperature for 19 h, then filtered, and evaporated in vacuo. The residue was dissolved in 200 mL of CH_2Cl_2. This dark brown solution was washed with 3 N sodium hydroxide (60 mL), 2 N sodium hydroxide (60 mL), and a saturated solution of NaCl, dried on sodium sulfate, and concentrated in vacuo to provide **22**: yield 1.24 g (60%); mp 136 °C. Anal. ($C_{10}H_9NO_2$) C, H, N.

1-(1-Acetyl-5-methoxyindol-2-yl)-2-(4-pyridyl)propene (23). Aldehyde **22** (3.38 g, 19.3 mmol) and 10 mL (4.5 × 19.3 mmol) of 4-ethylpyridine were dissolved in 93.5 mL of acetic anhydride, and the mixture was refluxed for 60 h. Acetic anhydride (63 mL) was then distilled under reduced pressure from the reaction mixture, and the solution was allowed to cool to room temperature. 3 N HCl (3 mL) was added, and stirring was continued for 10 min. The resulting suspension was filtered, 16.8 g of K_2CO_3 (final pH: 10) was added, and the aqueous filtrate was extracted with CH_2Cl_2 (3 × 100 mL). The extracts were washed with water (100 mL), dried over sodium sulfate, and evaporated in vacuo to give 7.5 g of the crude alkene. Chromatography over a silica gel column ($CHCl_3$–MeOH–H_2O, 9:1:0.1) afforded **23**: yield 0.58 g (10%); mp 214 °C. Anal. ($C_{19}H_{18}N_2O_2$) C, H, N.

10-Methoxy-5-methyl-7*H*-pyrido[4,3-*c*]carbazole (24). A solution of 0.57 g (1.85 mmol) of alkene **23** and 0.15 g of iodine in 560 mL of 95% ethanol was irradiated (Hanau TQ 150 high-pressure photoreactor) for 8 h. 1 N sodium hydroxide (4.2 mL), then 0.036 g of $Na_2S_2O_3 \cdot 5H_2O$, and finally 2000 mL of H_2O were added. The aqueous solution was extracted with ethyl acetate (600 mL). The organic extracts were dried over sodium sulfate and concentrated in vacuo to give **24**: yield 0.093 g (16%). The mother liquor was evaporated to dryness in vacuo. Chromatography of the residue over a silica gel column ($CHCl_3$–MeOH–H_2O, 9:1:0.1) afforded **24**: total yield 0.136 g (28%); mp 280 °C. Anal. ($C_{17}H_{14}N_2O$) C, H, N.

1-(5-Methoxyindol-2-yl)-2-(4-pyridinio)propene Methiodide (25). 4-Ethylpyridinium methiodide was prepared by reaction of methyl iodide on 4-ethylpyridine in ether, with an 87% yield. To a stirred solution of 1.03 g (4.14 mmol) of 4-ethylpyridinium methiodide in 7 mL of ether was added dropwise over 1 h a solution of 0.66 g (3.8 mmol) of 5-methoxyindole-2-carboxaldehyde (**22**) in 7 mL of piperidine and 5 mL of ether. The resulting suspension was stirred for 21 h at room temperature. The crystalline solid was collected by filtration, and **25** was obtained: yield 0.33 g (22%). Concentration of the mother liquor gave an additional 0.67 g of **25**: total yield 1 g (63%); mp >290 °C. Anal. ($C_{18}H_{19}IN_2O$) C, H, N, I.

10-Methoxy-5-methyl-7*H*-pyrido[4,3-*c*]carbazolium Methiodide (26). A solution of 0.5 g (1.23 mmol) of the alkene **25** in 1000 mL of EtOH was irradiated (Hanau TQ 150 high-pressure photoreactor) for 65 h with stirring. The reaction mixture was evaporated in vacuo, and the residue was chromatographed over a silica gel column ($CHCl_3$–MeOH, 7:3) to give **26**: yield 0.071 g (14%); mp >290 °C. Anal. ($C_{18}H_{17}IN_2O$) C, H, N, I.

Quaternization of 7*H*-Pyridocarbazoles by Methyl Iodide: 27, 28, 29, 30. The appropriate 7*H*-pyridocarbazole (1 mmol) was dissolved in 4 mL of DMF at 50 °C. Methyl iodide (5.3 mmol, 5.3 equiv) was added dropwise to the solution. The reaction mixture was stirred overnight at 50 °C and then cooled to room temperature, and the yellow crystalline precipitate was collected by filtration to give the desired 7*H*-pyridocarbazolium salts. **15** yielded **27** (87%): mp 240 °C. Anal. ($C_{18}H_{17}IN_2O$) C, H, N, I. **16** yielded **28** (63%): mp >290 °C. Anal. ($C_{18}H_{17}IN_2O$) C, H, N, I. **17** yielded **29** (17%): mp >290 °C. Anal. ($C_{18}H_{17}IN_2O$) C, H, N, I. **18** yielded **30** (77%): mp >290 °C. Anal. ($C_{19}H_{19}IN_2O$) C, H, N, I.

Quaternization of 7*H*-Pyridocarbazoles by *N*-(2-Chloroethyl)piperidine: 31, 32, 33. *N*-(2-Chloroethyl)-piperidinium hydrochloride (1.1 equiv) was dissolved in 0.5 mL of DMF and 0.2 mL of H_2O. This solution was added to a stirred solution of 1 equiv of pyridocarbazole monomers in 3 mL of DMF at 80 °C. The solution was stirred overnight at 80 °C. The resulting precipitate was collected by filtration and thoroughly washed with ether. After drying in vacuo, the pyridocarbazolium salt recrystallized from ethanol. **24** yielded **31** (35%): mp >290 °C. Anal. ($C_{24}H_{28}ClN_3O \cdot 1HCl$) C, H, N, Cl. **15** yielded **32** (55%): mp 220 °C. Anal. ($C_{24}H_{28}ClN_3O \cdot 1HCl$) C, H, N, Cl. **18** yielded **33** (10%): mp 184 °C. Anal. ($C_{25}H_{30}ClN_3O \cdot 1HCl$) C, H, N, Cl.

Quaternization with 1,1′-Bis(2-chloroethyl)-4,4′-bipiperidine: 34, 35, 36, 37, Dihydrochlorides. A solution of 0.6 equiv of 1,1′-bis(2-chloroethyl)-4,4′-bipiperidine in 2 mL of DMF

and 0.2 mL of H_2O was added dropwise over 2 h to a stirred solution of the corresponding pyridocarbazole (1 equiv, 0.1 g) in 3 mL of DMF. The mixture was stirred overnight at 80 °C, and the resulting crystalline solid was filtered, thoroughly washed with cold DMF and then with ether, and dried in vacuo to give about 20% of the desired dimer. The filtrate was evaporated to dryness in vacuo and chromatographed over a Sephadex LH20 resin column ($CHCl_3$–MeOH, 1:1). **24** yielded **34** (21%): mp >290 °C. Anal. ($C_{48}H_{54}Cl_2N_6O_2$·2HCl) C, H, N, Cl. **15** yielded **35** (60%): mp 273 °C. Anal. ($C_{48}H_{54}Cl_2N_6O_2$·2HCl) C, H, N, Cl. **18** yielded **36** (20%): mp >290 °C. Anal. ($C_{50}H_{58}Cl_2N_6O_2$·2HCl) C, H, N, Cl. **16** yielded **37** (12%): mp >290 °C. Anal. ($C_{48}H_{54}Cl_2N_6O_2$·2HCl) C, H, N, Cl.

Interaction with DNA. The K_{ap} values were determined at 25 °C in 0.1 M Tris·HCl, pH 7.4, 0.1 M NaCl buffer, by fluorescence measurements. Both excitation and emission wavelengths could be selected through a monochromator. Excitation light was provided by a xenon lamp. The temperature was 25 °C.

Monomers: Competition with Ethidium Bromide. Four cells were used simultaneously for each experiment. The first cell contained the buffer, the second one the DNA (4 μg/mL), and the third and fourth the drug plus DNA. The compounds were used at a concentration of 10^{-5} M. Microliter portions of ethidium bromide (100 μg/mL in buffer) were added. The fluorescence of ethidium bromide was excited at 546 nm and emission recorded at 590 nm. Scatchard plots of competition were calculated with a 9810-1 Hewlett-Packard calculator.

Dimers: Competition with Ethidium Dimer. The fluorescence of ethidium dimer synthesized in this laboratory[36] was excited at 540 nm and emission recorded at 610 nm. Ethidium dimer (6.4×10^{-7} M) and calf thymus DNA (base pair concentration: 1.6×10^{-6} M) were equilibrated for 24 h before measurements with different concentrations of the competing drug. The concentration of bound ethidium dimer per base pair was deduced from the fluorescent measurements and the complete displacement computed as described by Gaugain et al.[27] for different binding constants. The number n of DNA base pairs covered by the pyridocarbazole dimers is required to fit the experimental DNA displacing curves of the fluorescent ethidium dimer. It is obtained by competition with ethidium bromide.[27] The computed curves and experimental one are compared to evaluate K_{ap} constants.

Viscometric measurements were performed at 25 °C in semimicro dilution capillary viscometer with a suspended level (Cannon Instrument Co., State College, PA) mounted in a highly accurate thermostated bath. Flow times were measured to ±0.1 ms by the combined use of photoelectric sensors and an electronic timer.[37]

To measure the length increase of short DNA segments, the intrinsic viscosity of sonicated calf thymus DNA in the presence of an increasing concentration of agents was measured. The value of $\log [\eta]/[\eta_0]$ was plotted as a function of $\log (1 + 2r)$, where $[\eta]$ and $[\eta_0]$ are the intrinsic viscosities of the DNA measured in the presence and in the absence of bound agents, respectively, and r is the number of bound agents per nucleotide of DNA.[28] The slopes are interpreted in terms of mono- or bisintercalation (see discussion).

Drug Exposure and Cell Survival Determination. Exponentially growing L1210 cells (1×10^5/mL) were incubated for 24 h at 37 °C in RPMI 1640 supplemented with fetal calf serum (10%) medium containing different drug concentrations.[13] Total cell number in the different cultures was determined by means of a Cytograf Model 6300 A instrument (Bio/Physics Systems, Inc.). The average number of cells in each duplicate treated culture was expressed as a percentage of the average number of cells in the triplicate untreated controls. The ED_{50} value was obtained by plotting the percentage of residual cells vs. the drug concentration on a semilogarithmic scale. The ED_{50} was determined from a linear regression.

For the determination of the cloning efficiency,[13] the cells treated for 24 h were diluted in RPMI 1640 to concentrations of 250 and 500 cells/mL; 2.5 mL of suspension was then added to 0.4 mL of Noble agar (Difco Laboratories) solution before plating on 35-mm-diameter Petri dishes. After 14 days of incubation at 37 °C in an atmosphere of 5% carbon dioxide humidified air, colonies larger than 0.2 mm in diameter were counted by means of a Biotran III counter (New Brunswick Co., Inc). Triplicate assays were carried out at each drug concentration. The cloning efficiency in the controls was about 65%. Dose–response curves were used to determine the mean lethal concentration (CE_{37}), defined as the concentration required to reduce cloning efficiency to a factor of 0.37.

Biological Testing. L1210 cells (10^5) were inoculated intraperitoneally into DBA_2 mice. There were 20–30 animals/group. Twenty-four hours later, one group was treated with an intraperitoneal inoculum of the studied drug and another group (control group) received the same volume of the solvent used to dissolve the compound under study. Deaths were recorded every day at the same hour. Animals that survived for more than 45 days were considered cured. The mean survival time of treated animals (T) was compared to that of control animals (C). The increases in life span (ILS) were calculated as $T/C \times 100$.

The acute toxicity was determined by the usual procedure. OD is the optimal dose. MTD is the highest dose that could be administered without causing animal death. They are expressed in milligrams/kilogram. The statistical significance of the results was determined by using Student's test.

Acknowledgment. We are very grateful to Dr. R. Oberlin, J. Couprie, and S. Guerineau for their technical assistance, to Dr. A. Gouyette for mass spectra recording, and to A. Bouju for typing the manuscript. This work was supported by funds from the Ligue Nationale Française contre le Cancer, the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, and the Université René Descartes.

Registry No. 1, 75525-73-4; **2**, 109960-18-1; **3**, 109960-19-2; **4**, 109960-20-5; **5**, 109960-21-6; **6**, 75525-72-3; **7**, 109960-22-7; **8**, 109960-23-8; **9**, 109056-40-8; **10**, 109056-41-9; 11, 109056-42-0; **12**, 109960-24-9; **13**, 109960-25-0; **14**, 109960-26-1; **15**, 109056-19-1; **16**, 63081-07-2; **17**, 109056-20-4; **18**, 109056-22-6; **19**, 4382-54-1; **20**, 4792-58-9; **21**, 21778-77-8; **22**, 21778-81-4; **23**, 109960-27-2; **24**, 109056-21-5; **25**, 109960-28-3; **26**, 109960-29-4; **27**, 109960-30-7; **28**, 109960-31-8; **29**, 109960-32-9; **30**, 109960-33-0; **31**, 109960-34-1; **32**, 109960-35-2; **33**, 109960-36-3; **34**, 109056-35-1; **35**, 109056-34-0; **36**, 109960-37-4; **37**, 109056-36-2; **38**, 62099-82-5; **39**, 62099-83-6; **40**, 62099-80-3; 41, 62099-79-0; **42**, 109960-38-5; **43**, 75413-46-6; **44**, 75413-48-8; **45**, 105823-48-1; **46**, 74517-54-7; **47**, 74517-51-4; **48**, 74517-52-5; 2-[(dimethylamino)methyl]-5-methoxyindole methiodide, 62099-67-6; 2-formylpyridine, 1121-60-4; 3-formylpyridine, 500-22-1; 4-formylpyridine, 872-85-5; 4-ethylpyridine, 536-75-4; 4-ethylpyridinium methiodide, 32188-16-2; *N*-(2-chloroethyl)piperidinium hydrochloride, 2008-75-5; 1,1′-bis(2-chloroethyl)-4,4′-bipiperidine, 6802-93-3.

(36) Gaugain, B.; Barbet, J.; Oberlin, R.; Roques, B. P.; Le Pecq, J. B. *Biochemistry* **1978**, *17*, 5071.
(37) Le Pecq, J. B. *Methods Biochem. Anal.* **1971**, *20*, 41.