Asymmetrical Bisintercalators as Potential Antitumor Agents

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Ditercalinium and its analogues are dimeric molecules made up of two identical 7*H*-pyrido[4,3-*c*]carbazole rings linked by symmetrical linking chains. These dimers elicit antitumor properties through a new mechanism of action. Recently, a relationship was found between their antitumor properties and their cytotoxic effect on the *polA Escherichia coli* mutant strain, suggesting that 7*H*-pyrido[4,3-*c*]carbazole dimers might induce a DNA deformation that could be recognized by the *E. coli* SOS repair system. Thus, the role of symmetry in ditercalinium analogues for their DNA binding, antitumor properties, and bacterial toxicity is investigated in the present study, by introducing asymmetric parameters in their structures. Dimers were either synthesized with an asymmetrical rigid linking chain or made up of two chemically different chromophores, i.e., acridine and 7*H*-pyrido[4,3-*c*]carbazole. The asymmetrical dimers remain able to bisintercalate into DNA with high affinities, but a dramatic loss in their antitumor potency is observed. On the other hand, these asymmetrical dimers are cytotoxic for *polA E. coli* mutants, like their symmetrical analogues. These results show that the symmetry plays a crucial role for the antitumor potency in the 7*H*-pyrido[4,3-*c*]carbazole dimers series.

In order to obtain new compounds endowed with high DNA affinity, different polyfunctional intercalators have been synthesized (review in ref 1). As expected these oligomeric compounds bind to DNA with very high affinities, reaching in the case of acridine trimers[2] those of DNA regulatory proteins.[3,4] Among these molecules, the 7*H*-pyrido[4,3-*c*]carbazole dimers exhibit DNA binding properties and antitumor activities that are greatly dependent on their structure.[5-7] Thus, a rigid bis(ethyl)bipiperidine linking chain that prevents intramolecular self-stacking of the two heterocyclic rings is an important requirement for DNA bisintercalation.[5] Moreover, the occurrence of strong antitumor properties is strictly associated with the location of the quaternarized nitrogen in position 2 of the pyridinic ring.[5,6] One of these compounds, ditercalinium (NSC 366241), presently under clinical trial, has a mechanism of action that appears to be completely different from that of its corresponding monomers and other mono- or bisintercalators.[8]

Ditercalinium was recently shown, by NMR studies, to bisintercalate with its linking chain lying in the major groove of the self-complementary tetranucleotide d-$(CpGpCpG)_2$.[9] This contrasts with the DNA binding of other bisintercalators such as the quinoxaline antibiotics, which bisintercalate into DNA with their peptidic dimerization chain located in the minor groove.[10]

The antitumor potency of symmetrical dimers derived from ditercalinium is greatly dependent on the length of the dimerization chain.[7] Therefore, the mechanism of action of these dimers in vivo has been assumed to be mainly related to a lethal drug-induced DNA deformation.[11]

Following this hypothesis, the role of symmetry in the antitumor potency of these dimers was investigated by synthesizing new compounds bearing an asymmetrical rigid linking chain or made up of two chemically different intercalating moieties. The acridine ring was chosen as the planar aromatic moiety to be linked to the 7*H*-pyrido-[4,3-*c*]carbazole or to its 7-methylated derivative on the basis of its size, roughly similar to that of the 7*H*-pyridocarbazole, to its well-known intercalating ability,[12,13] and to the occurrence of antitumor potency in some acridine dimers series.[14,15] This latter substitution was shown to reinforce the antitumor potency in the symmetrical dimers[7] series.

The synthesis, DNA binding, and antitumor properties, as well as the effect on *Escherichia coli* strains of these two series of dimers, are reported in this paper.

Chemistry

Asymmetrical Dimers. To obtain the asymmetrical 7*H*-pyrido[4,3-*c*]carbazole dimers **5** and **6**, the synthesis of the asymmetrical dimerization spacer **4** was first required. This linking chain was prepared according to Scheme I. Monoquaternarization of 4,4'-bipyridine by 1 equiv of 2-bromoethanol afforded compound **1**. Several attempts to directly quaternarize the free nitrogen of the monoquaternarized bipyridine **1** by 3-bromopropanol failed. Since asymmetrical disubstitution of bipiperidine derivatives has been reported,[16] compound **1** was hydrogenated over PtO_2 to give the corresponding monosubstituted bipiperidine derivative **2**. This compound was then substituted by 3-bromopropanol to provide the asymmetrically disubstituted intermediate **3**. Reaction of this latter molecule with thionyl chloride via the usual

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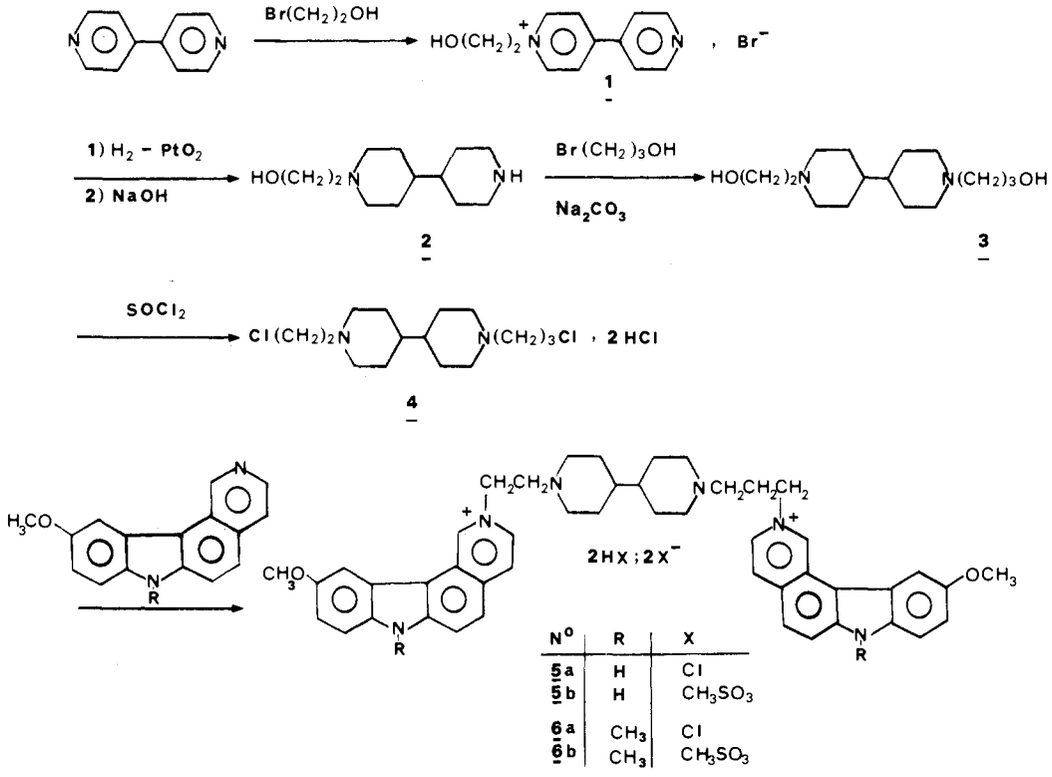
Scheme I. Synthesis of the 7*H*-Pyrido[4,3-*c*]carbazole Dimers with Asymmetrical Linking Chain



procedure[5] yielded the desired asymmetrical linking chain **4**. The two 7*H*-pyrido[4,3-*c*]carbazole dimers **5a** and **6a** were then obtained by direct condensation of 2 equiv of the appropriate heterocycle with 1 equiv of the linking chain **4** as previously described.[5] However, whereas dimerization of 7*H*-pyrido[4,3-*c*]carbazole rings with symmetrical chains afforded good yields of pure dimers with only reduced amounts of monomeric species,[5-7] the condensation of the asymmetrical chain **4** gave only poor yields of the expected dimers. Moreover, two successive purification steps by exclusion and silica gel chromatography were required to discard byproducts. One of these byproducts, whose structure was established by NMR and mass spectral analyses, results from the condensation of only one 7*H*-pyridocarbazole ring with the linking chain (data not shown). Conversion of dimers **5a** and **6a** into tetramethyl sulfonate salts **5b** and **6b** was performed by reaction with CH_3SO_3Ag via the previously described procedure.[7]

7*H*-Pyrido[4,3-*c*]carbazole–Acridine Heterodimers. Two different synthetic pathways were investigated, starting either from the 7*H*-pyridocarbazole ring by addition of the linking chain followed by condensation of the acridine or from the acridine ring with use of an inverse pathway. However, several attempts to condense the 7*H*-pyridocarbazole ring with 1-(2-chloroeth-1-yl)-4,4′-bipiperidine dihydrochloride (obtained from **2** by reaction with $SOCl_2$) were unsuccessful. Therefore, the synthetic pathway starting from 6,9-dichloro-2-methoxyacridine was followed, as summarized in Scheme II. The acridine derivative **8** was easily obtained from the 6,9-dichloro-2-methoxyacridine by substitution with 2-aminoethanol in phenol,[17] followed by substitution of the hydroxyl group by a chloro group with thionyl chloride. Substitution of the acridine derivative **8** with the asymmetrical bipiperidine chain **2** afforded the monomer **9**, which was subsequently transformed into its hydrochloride derivative **10**·3HCl with thionyl chloride. Attempts to quaternarize the 7*H*-pyrido[4,3-*c*]carbazole ring by the positively charged derivative **10**·3HCl failed, and acridone, resulting from elimination of the bipiperidine chain, was essentially obtained. This degradation was assumed to result from the presence of positive charges on the piperidinic rings, and therefore this charged molecule **10**·3HCl was neutralized with use of mild conditions (NH_4OH). Quaternarization of the 7*H*-pyridocarbazole ring with the neutral acridine derivative **10** gave the dimers **11a** and **12a** in good yields. In order to enhance their water solubility, transformation of dimers **11a** and **12a** into their dimethanesulfonate salts **11b** and **12b** was performed by reaction of these compounds with 2 equiv of methanesulfonic acid.

Interaction with DNA. The DNA binding affinity of the dimers was measured by using the previously proposed fluorometric method,[18,19] and their DNA intercalating ability was determined by viscometry as already reported.[5] According to this method, the plot of log (η/η_0) versus log $(1 + 2r)$ (see the Experimental Section) gives a slope ΔL ranging from 4 to 6 for bisintercalators and 2 to 3 for monointercalating ligands.[20] The effect of the asymmetrical dimers on the thermal helix–coil transition of poly[d(AT)] was also studied by UV absorption (260 nm) measurements performed at a base pair to dye ratio of 13.

The properties of the asymmetrical dimers **5b**, **6b**, **11b**, and **12b** are presented in Table I and are compared to those obtained for previously described[5,10] symmetrical 7*H*-pyrido[4,3-*c*]carbazole dimers. The asymmetrical dimers **5b**, **6b**, **11b**, and **12b** elicit high DNA affinities (10^7–10^8 M^{-1}) lying in the same range as those observed

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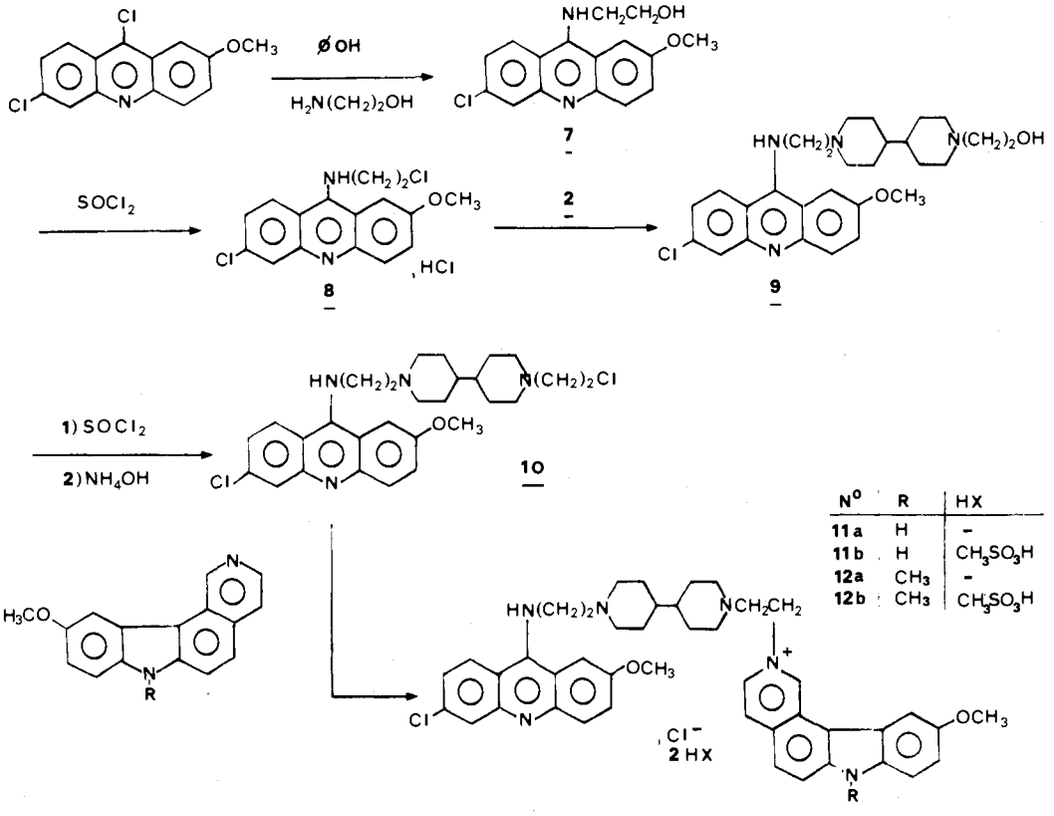
Scheme II. Synthesis of 7*H*-Pyrido[4,3-*c*]carbazole–Acridine Heterodimers



for the symmetrical dimers (**13–20**). Such a high DNA affinity was also found for a 9-amino-6-chloro-2-methoxyacridine dimer bearing the rigid bis(ethyl)bipiperidine linking chain (unpublished results). On the other hand, except for dimer **6b**, the asymmetrical compounds bisintercalate into DNA as shown by their ΔL values lying between 4 and 6. The rather weak enhancement in DNA lengthening (ΔL = 3) induced by dimer **6b** is related to its weak solubility in presence of DNA at pH 7.4.

Thus, introducing asymmetrical parameters in 7*H*-pyrido[4,3-*c*]carbazole dimers does not influence their DNA binding properties since they remain able to bisintercalate into DNA with high affinity.

On the other hand, concerning the 7*H*-pyridocarbazole–acridine heterodimers, NMR studies have recently shown that like ditercalinium, symmetrical acridine dimers bearing a (aminocarbonyl)alkyl spacer bisintercalate into oligonucleotides with their rather rigid linking chain lying into the major groove.[21] The DNA bisintercalation of heterodimers **11b** and **12b** is consistent with these findings, but contrasts with the mode of DNA binding of an ethidium–acridine heterodimer previously synthesized in our laboratory.[22] Indeed, this dimer was shown to intercalate only one of its chromophores, the phenanthridinium ring, into DNA with the linking chain assumed to lie in the minor groove.[18]

The shift toward high temperatures induced by the asymmetrical dimers on the thermal helix–coil transition of poly[d(AT)] is in agreement with their high DNA binding affinity. All melting curves are biphasic (data not shown), a result already observed with compounds covering several base pairs.[23] The T_m values obtained with the asymmetrical dimers lie in the same range as those observed with their symmetrical analogues.[7]

In conclusion, as recently shown with symmetrical 7*H*-pyrido[4,3-*c*]carbazole dimers bearing linking chains of different length,[7] the DNA affinity constants and intercalating ability of the asymmetrical molecules studied here are almost independent of the structural modifications introduced in the spacer. Moreover, modification of the nature of one of the intercalating units does not influence the DNA binding properties of these molecules.

Antitumor Properties. The cytotoxicity and antitumor activity of the four asymmetrical dimers were determined on L1210 murine leukemia because of its good predicting value for activity in human cancer.[24]

The results obtained with these new compounds (**5b**, **6b**, **11b**, **12b**) are compared to those previously reported for symmetrical 7*H*-pyrido[4,3-*c*]carbazole dimers. In a recent study,[7] we found that increasing the length of the linking chain of ditercalinium (**13**) by one or two methylenes (**15–18**) does not induce large changes in the antitumor properties of the corresponding dimers which elicit T/C values ranging from 148 to 190 (Table I). However, a further increase in the length of the spacer dramatically reduces the antitumor potency of the 7*H*-pyridocarbazole dimers (**19**, **20**).

As shown by the biological results reported in Table I, asymmetrical dimers **5b** and **6b** elicit small or no antitumor properties in vitro and in vivo. This result emphasizes again that a high DNA affinity is required for antitumor activity but that this property alone is not sufficient, an observation already reported in other 7*H*-pyridocarbazole dimers series.[5-7] Moreover, dimers **5b** and **6b**, which have

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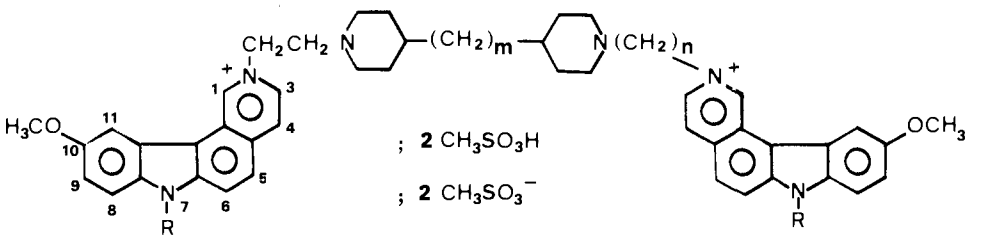
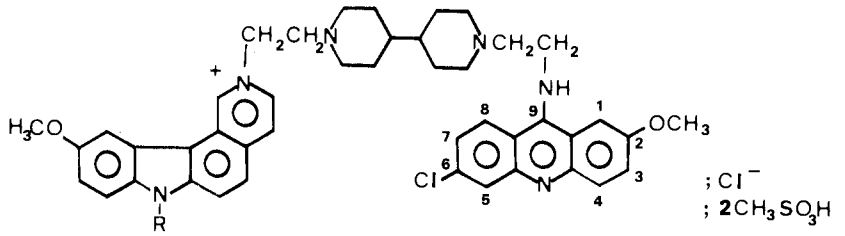
Table I. Asymmetrical Bisintercalators: DNA Binding Properties, Antitumor Activities, and Cytotoxicity on *E. coli*[a]



no.	R	*m*	*n*	ΔL[a]	$K_{ap} \times 10^{-7}$ M^{-1}[b]	T_m, °C[c]	ED_{50},[d] μM	EC_{37},[e] μM	MTD,[f] mg/kg	OD,[g] mg/kg	T/C, %[h]	*polA*:[i] EC_{37}, μM
5b	H	0	3	4.25	5	83	0.72	0.090	20	10	137	0.016
6b	CH_3	0	3	~3[k]	1	67	>0.9	0.112	10	10	128	0.025
13[b,c]	H	0	2	3.60	1	80.8	0.19	0.024	10	10	182	0.013
14[c]	CH_3	0	2	3.60	2	ND	0.36	0.006	10	2.5	178	0.013
15[d]	H	1	2	5.23	10	77.4	1.08	0.063	30	7	175	0.021
16[d]	CH_3	1	2	5.28	20	ND	0.70	0.013	10	3	190	0.014
17[d]	H	2	2	4.83	7	78.5	1.34	0.22	20	5	143	0.015
18[d]	CH_3	2	2	3.56	3	ND	0.9	0.77	10	5	170	0.225
19[c]	H	3	2	5.58	5	ND	0.34	0.14	10	5	NS	0.087
20[c]	CH_3	3	2	7	20	ND	0.26	0.15	10	10	NS	0.009



no.	H	ΔL[a]	$K_{ap} \times 10^{-7}$ M^{-1}[b]	T_m, °C[c]	ED_{50}, μM[d]	EC_{37}, μM[e]	MTD, mg/kg[f]	OD, mg/kg[g]	T/C, %[h]	*polA*:[i] EC_{37}, μM
11b	H	5.06	5	77	2.7	17	15	15	NS	0.057
12b	CH_3	4.45	10	69.2	0.24	>0.9	15	15	NS	0.029

[a] DNA binding properties: (a) Slope of the plot of log (η/η_0) versus log $(1 + 2r)$. (k) Precipitation of the dimer prevented precise viscometric measurement at this pH. (b) Affinity constant expressed in inverse molarity. (c) Melting temperature measured for a 50% fraction coil. The poly[d(AT)] transition alone is at 68.1 °C. ND: not determined. Cellular toxicity: (d) Dose that inhibits 50% of the cell growth after 24-h exposure to the drug. (e) Dose required to inhibit the cloning efficiency to a factor of 37%. Antitumor activity in vivo on L1210-infected mice: (f) Maximal tolerated dose. (g) Optimal dose. (h) Treated mean survival time per control mean survival time. NS: nonsignificant value. Cytotoxicity on *E. coli* strains: (i) Cytotoxicity on *polA* strain mutant expressed in EC_{37} (dose required to inhibit 37% of the cell growth). All the compounds are devoid of cytotoxicity for the wild-type *E. coli* strain ($EC_{37} > 1$ μM). [b] Ditercalinium. [c] From ref 5. [d] From ref 10.

linking chains identical in length with those of the highly active dimers **15** and **16**, are inactive on L1210 murine leukemia. This result suggests that the loss of antitumor activity of dimers **5b** and **6b** could be related either to changes in the conformation of their linking chain or to the loss of symmetry of these compounds.

It has previously been shown that a pharmacological activity is related to the ability of the symmetrical dimers to induce a DNA distorsion.[7] The present results suggest that asymmetrical dimers might induce a different DNA deformation or that they do not modify significantly the DNA structure upon intercalation. Finally it is noticeable that, in contrast with the symmetrical series, introduction of a methyl group in position 7 of the intercalating ring does not provide active compounds in the asymmetrical series.

Although they bisintercalate into DNA with high affinity, the 7*H*-pyrido[4,3-c]carbazole–acridine heterodimers **11b** and **12b** are completely devoid of antitumor activity. This result strengthens the finding that a drastic reduction in antitumor potency is observed upon introduction of asymmetrical parameters in the structure of 7*H*-pyridocarbazole dimers. However, whether or not the loss of antitumor activity of dimers **11b** and **12b** is only related to their asymmetrical structure cannot be yet ascertained.

Effect on *E. coli* Bacterial Strains. In a recent study, Lambert et al.[11] have shown a relationship between the antitumor activity of ditercalinium and its cytotoxicity on *E. coli* mutant strains. A bacterial mutant strain specifically sensitive to ditercalinium was isolated, and the mutation responsible for sensitivity to this drug was characterized and found to be the *polA* mutation. The authors proposed that ditercalinium, by inducing a DNA conformational change similar to that caused by covalent adducts, would act for the UV repair system as a dummy lesion. Attempts to repair this dummy lesion would lead to an abortive repair process lethal for *polA* mutants. Moreover, it was suggested that the structure of the linking chain of ditercalinium might play a crucial role in the DNA deformation recognized by the UV repair system. Therefore, as a first step, the cytotoxicity of symmetrical 7*H*-pyridocarbazole dimers with linking chains or various lengths (**13–20**) was tested on both wild type and *polA E. coli* strains. The results obtained were related to the antitumor properties of the dimers.

Preliminary results presented in Table I show that symmetrical dimers bearing short linking chains (**15–17**) behave similarly to ditercalinium. Indeed these compounds are cytotoxic for the *polA* strain and display high antitumor activity. In contrast, dimer **18**, which also elicits high antitumor properties, is much less cytotoxic for the *polA* strain. This result suggests that this compound might not induce the DNA deformation recognized by the UV repair system and lethal for *polA* mutants but that it might

express its antitumor properties through a mechanism of action different from that of ditercalinium.

Compounds **5b** and **6b** with asymmetrical linking chains display little antitumor activity but elicit high cytotoxic effect on *polA* strain. Similarly, the acridine–7*H*-pyrido[4,3-*c*]carbazole heterodimers are cytotoxic for the *polA* strain but are completely devoid of antitumor potency. Dimers **19** and **20** also show such a behavior since they are cytotoxic on *polA* strain and devoid of activity on L1210 murine leukemia.

These results reinforce the assumption that the cytotoxicity for the *polA* strain might be closely related to the nature and length of the linking chain responsible for a DNA conformational change.

By contrast, the antitumor potency appears to be dependent on both the size and symmetry of the spacer and the nature of the intercalating chromophores.

Experimental Section

Melting points were determined on a Kofler apparatus and were not corrected. The structure of the compounds was confirmed by ^{1}H NMR spectroscopy (Bruker WH 270 MHz) in Me_2SO-d_6. The purity was checked by thin-layer chromatography on silica gel plates (Merck) with the following solvents (v/v): R_f (A), 1-BuOH/Pyr/AcOH/H_2O (5:2:1:2), R_f (B), 2-PrOH/NH_4OH (7:3), R_f (C), 1-BuOH/NH_4OH (17:5), R_f (D), CH_2Cl_2/MeOH (9:1). Mass spectra were performed on a double focusing VG 7°-250 spectrometer, (VG Instrument), equipped with a fast atom bombardment (FAB) gun (Ion Tech.) and are given only for the final compounds (dimers). Where analyses are indicated only by the symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

10-Methoxy-7-methyl-7*H*-pyrido[4,3-*c*]carbazole was synthesized according to Pelaprat et al.[25] 4,4′-Bipyridine and 6,9-dichloro-2-methoxyacridine were commercially available from Aldrich. The following abbreviations are used: MeOH, methanol; EtOH, ethanol; 1-BuOH, 1-butanol; 2-PrOH, 2-propanol; AcOH, acetic acid; Me_2SO-d_6, hexadeuteriodimethyl sulfoxide; HMDS, hexamethyldisiloxane; Et_2O, diethyl oxide; DMF, dimethylformamide; FAB, fast atom bombardment.

1-(2-Hydroxyeth-1-yl)-4,4′-bipyridine Bromide (1). 2-Bromoethanol (2.27 mL, 32 mM) was added to a stirred solution of 5 g (32 mM) of 4,4′-bipyridine in 200 mL of Et_2O. The mixture was stirred at room temperature for 18 h, and the resulting crystalline suspension was filtered, yielding 0.8 g (2 mM; 6% yield) of 1,1′-(2-hydroxyeth-1-yl)-4,4′-bipyridinium dibromide as white crystals. The filtrate was evaporated in vacuo, and the residue was solubilized in 20 mL of anhydrous acetone. The solution was refluxed for 30 h. After cooling, the white crystals formed were collected by filtration, washed with acetone, and dried in vacuo. **1**: yield 9.3 g (80%); mp 212 °C; R_f 0.27 (A). Anal. ($C_{12}H_{13}BrN_2O$) C, H, N, Br.

1-(2-Hydroxyeth-1-yl)-4,4′-bipiperidine (2). A 2.4-g (6.6-mM) portion of **1** was solubilized in 100 mL of a mixture H_2O/EtOH (50:50). PtO_2 (0.4 g) as catalyst and 4.5 g (4 equiv) of a 48% solution of aqueous HBr were added. The mixture was hydrogenated under pressure (55 kg) for 10 h. The catalyst was filtered, and the filtrate was evaporated in vacuo. Five milliliters of H_2O and 20 mL of 1 N NaOH were added to the oily residue, and the solution was extracted with CH_2Cl_2 (5 × 30 mL). The organic extracts were dried over Na_2SO_4 and evaporated in vacuo. **2**: yield 1.26 g (90%); mp 144 °C; R_f 0.12 (A). Anal. ($C_{12}H_{24}N_2O$) C, H, N.

1-(2-Hydroxyeth-1-yl)-1′-(3-hydroxyprop-1-yl)-4,4′-bipiperidine (3). One gram (4.7 mM) of **2** was solubilized in 60 mL of anhydrous EtOH. Na_2CO_3 (0.5 g, 4.7 mM) was added. The mixture was heated to 70 °C, and solution of 0.57 g (1.4 × 4.7 mM) of 3-bromopropanol in 10 mL of anhydrous EtOH was added dropwise over 2 h. The solution was refluxed for 4 days. The crystalline suspension was filtered, and the filtrate was evaporated in vacuo. Recrystallization of the residue in EtOH gave 1.7 g of **3**: yield 1.7 g (83%); mp 220 °C; R_f 0.56 (B). Anal. ($C_{15}H_{30}N_2O_2$) C, H, N.

1-(2-Chloroeth-1-yl)-1′-(3-chloroprop-1-yl)-4,4′-bipiperidine Dihydrochloride (4). Thionyl chloride (7.6 mL, 30 × 3.5 mM) was added to a suspension of 1.5 g (3.5 mM) of **3** in 200 mL of chloroform. The mixture was refluxed for 2 h and then allowed to cool to room temperature, the toluene was removed in vacuo, and the white residue was thoroughly washed with Et_2O to remove excess thionyl chloride. **4**: yield 0.96 g (72%); mp >260 °C; R_f 0.91 (B). Anal. ($C_{15}H_{28}N_2Cl_2$·2HCl) C, H, N, Cl.

Dimerization with 1-(2-Chloroeth-1-yl)-1′-(3-chloroprop-1-yl)-4,4′-bipiperidine Dihydrochloride: 5a·2HCl and 6a·2HCl. A solution of 0.2 g (5.3 mM) of **4** in 2 mL of DMF–H_2O (80:20) was added dropwise over 1 h to a solution of 2 equiv (10.6 mM) of the corresponding 7*H*-pyridocarbazole in 5 mL of DMF. The mixture was heated at 80 °C for 24 h and then cooled to room temperature. The resulting solid was filtered, washed with cold DMF and then with Et_2O, and dried in vacuo to give 10% of the desired dimers **5a** and **6b**. The filtrate was evaporated in vacuo to dryness and chromatographed first over a Sephadex LH20 resin column (CH_2Cl_2–MeOH, 1:1) and then over a silica gel column (flash chromatography; BuOH–NH_4OH, 17:5). **5a**: total yield 20%. **6a**: total yield 14%.

Conversion into Tetramethanesulfonate: 5b·2CH$_3$SO$_3$H and 6b·2CH$_3$SO$_3$H. One equivalent of each dimer **5a** and **6a** as chloride was dissolved under reflux in 10 mL of EtOH–H_2O (20:80), and 4.02 equiv of CH_3SO_3Ag was added. After cooling, 0.02 equiv of KCl (10^{-2} M aqueous solution) was added to precipitate excess CH_3SO_3Ag. The mixture was centrifugated (five times, 20 min at 4000 rpm) and the residues discarded. The supernatant solutions were lyophilized to provide **5b** and **6b** as tetramethanesulfonate salts with 95% yield. **5b**: mp 210 °C; R_f 0.07 (C); FAB-MS (M^+) 732.42. Anal. ($C_{49}H_{58}N_6O_8S_2$·2CH$_3$SO$_3$H) C, H, N, S. **6b**: mp 170 °C; R_f 0.12 (C); FAB-MS (M^+) 760.72. Anal. ($C_{51}H_{62}N_6O_8S_2$·2CH$_3$SO$_3$H) C, H, N, S.

1-[(6-Chloro-2-methoxy-9-acridinyl)amino]-2-hydroxyethane (7). 6,9-Dichloro-2-methoxyacridine (0.3 g, 1.08 mM) was solubilized in 2.5 mL of phenol at 80 °C. 2-Aminoethanol (0.066 g, 1.08 mM) was added, and the solution was heated at 120 °C for 1.5 h under nitrogen. After being cooled to room temperature, the mixture was poured into 20 mL of Et_2O and 10 mL of ethyl acetate. The yellow precipitate was collected by filtration, washed with ethylacetate, and dried in vacuo. **7**: yield 0.29 g (90%); mp >260 °C; R_f 0.15 (D). Anal. ($C_{16}H_{15}N_2O_2Cl$) C, H, N, Cl.

1-[(6-Chloro-2-methoxy-9-acridinyl)amino]-2-chloroethane Hydrochloride (8). To a stirred suspension of 0.25 g (0.83 mM) of **7** in 5 mL of dry toluene was added 2.2 mL (36 × 0.83 mM) of thionyl chloride. The mixture was heated at 80 °C for 7 h. The resulting yellow crystals were filtered, washed with Et_2O, and dried in vacuo. **8**: yield 0.22 g (83%); mp >260 °C; R_f 0.60 (C). Anal. ($C_{16}H_{14}N_2OCl_2$·1HCl) C, H, N, Cl.

1-(2-Hydroxyeth-1-yl)-1′-[2-[(6-chloro-2-methoxy-9-acridinyl)amino]eth-1-yl]-4,4′-bipiperidine (9). A solution of 0.30 g (2.5 × 0.56 mM) of **2** in 20 mL of EtOH was added dropwise over 5 h to a stirred solution of 0.2 g (0.56 mM) of **8**. The mixture was refluxed for 3 days in the dark. The solution was concentrated in vacuo, and the residue was dried in vacuo. Recrystallization from MeOH–Et_2O afforded 0.26 g (yield 93%) of **9** as yellow crystals: mp 208 °C; R_f 0.46 (C). Anal. ($C_{28}H_{37}N_4O_2Cl$) C, H, N, Cl.

1-(2-Chloroeth-1-yl)-1′-[2-[(6-chloro-2-methoxy-9-acridinyl)amino]eth-1-yl]-4,4′-bipiperidine Trihydrochloride (10·3HCl). To a stirred suspension of 0.22 g (0.44 mM) of **9** in 5 mL of dry toluene was added dropwise at 80 °C 0.32 mL (10 × 0.44 mM) of pure thionyl chloride. The suspension was refluxed for 6 h and allowed to cool to room temperature overnight. The yellow crystals were collected by filtration, thoroughly washed with Et_2O, and dried in vacuo. **10**·3HCl: yield 0.17 g (63%); mp >260 °C; R_f 0.94 (C).

1-(2-Chloroeth-1-yl)-1′-[2-[(6-chloro-2-methoxy-9-acridinyl)amino]eth-1-yl]-4,4′-bipiperidine (10). A 0.17-g (0.27-mM) portion of 10·3HCl was solubilized in 10 mL of H_2O. NH_4OH (2 M aqueous solution) (0.54 mL, 4 × 0.27 mM) was added, and the solution was extracted with CH_2Cl_2 (8 × 10 mL). The organic extracts were dried over Na_2SO_4 and evaporated in

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vacuo, yielding 0.10 g (75%) of **10**: mp 240 °C. Anal. ($C_{28}H_{36}N_4Cl_2O$) C, H, N, Cl.

Quaternarization of 7*H*-Pyrido[4,3-*c*]carbazole with 10:11a and 12a. A 0.08-g (0.16-mM; 1-equiv) portion of **10** was solubilized in 2.5 mL of DMF. This solution was added dropwise to a stirred solution of 1.1 equiv of the appropriate 7*H*-pyrido-[4,3-*c*]carbazole in 2 mL of DMF. The solution was heated at 80 °C under nitrogen for 20 h. The resulting suspension was allowed to cool to room temperature and then evaporated to dryness in vacuo. The residue was flash chromatographed over a silica gel column (CH_2Cl_2–MeOH–NH_4OH, 8:2:0.5) and gave **11a**: yield 0.031 g (27%); R_f 0.11 (C). **12a**: yield 0.020 g (17%); R_f 0.32 (C).

Conversion of 11a and 12a into Their Dimethanesulfonate Salts 11b and 12b. One equivalent of **11a** or **12a** was solubilized in 3 mL of MeOH. Methanesulfonic acid (2 equiv) was added, and the solution was diluted with water (50 mL) and lyophilized. **11b**: yield 0.039 g (99%); mp 250 °C; FAB-MS (M^+) 727.35. **12b**: yield 0.025 g (99%); mp 206 °C; FAB-MS (M^+) 741.61.

Interaction with DNA. The K_{ap} values were determined at 25 °C in 0.1 M Tris·HCl, 0.1 M NaCl buffer pH 7.4, by fluorescence measurements based upon competition with ethidium dimer synthesized in our laboratory.[20] Excitation and emission were selected through a monochromator. The fluorescence of ethidium dimer was excited at 540 nm and emission recorded at 610 nm. Ethidium dimer (6.4×10^{-7} M) and calf thymus DNA (base pairs concentration: 1.6×10^{-6} M) were equilibrated for 24 h before measurements with different concentrations of drug. The concentration of bound ethidium dimer per base pair was deduced according to Gaugain et al.[16] The displacement curves were computed as described[16] and were compared to the experimental ones to evaluate the K_{ap} values. Viscometric measurements were performed at 25 °C by using the procedure already reported.[25] The intrinsic viscosity η of sonicated calf thymus DNA was measured in the absence (η_0) and presence (η) of increasing concentrations of drug. Plotting log (η/η_0) versus log ($1 + 2r$) where r is the number of bound ligand per nucleotide of DNA gives a slope ΔL, which accounts for monointercalation (ΔL between 2 and 3) or bisintercalation (ΔL between 4 and 6).

Biological Testing. Exponentially growing L1210 cells were treated with different drug concentrations.[25] Determinations of the dose effective in inhbiting 50% of the cell growth after 24-h exposure to the drug (ED_{50}) and of the dose required to inhibit the cloning efficiency to a factor of 0.37 after 24-h exposure to the drug (EC_{37}) were performed by using the method described in detail in our preceding paper.[6] Increases in life span (ILS) expressed as T/C × 100 values of DBA_2 mice inoculated with L1210 cells (10^5) were determined in the same way as reported elsewhere.[25] The statistical significance of the results was determined by the Student's t test.

Cytotoxicity Measurements. Bacteria were grown as reported in ref 11 and treated with various concentrations of the studied compounds. After 120 min of incubation at a temperature depending on the thermosensitivity of the *polA* mutation, bacteria were plated on LBT plates. Colonies were counted after an overnight incubation at 37 °C. This procedure was reported in detail by Lambert et al.[11]

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Supplementary Material Available: The NMR spectrum of heterodimer **11a** in Me_2SO-d_6 (in the presence of trifluoroacetic acid) is shown in Figure 1 (1 page). (Assignment of the protons was performed by using ^1H NMR COSY experiments.) Ordering information is given on any current masthead page.

Ara-tiazofurin: Conservation of Structural Features in an Unusual Thiazole Nucleoside

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Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC 286193) is a *C*-glycosyl thiazole nucleoside with antitumor activity. Crystal structures of tiazofurin and its α,2′-deoxy and xylo analogues all show close contacts between the thiazole sulfur (S) and the furanose ring oxygen (O1′). These contacts have been interpreted in terms of an attractive intramolecular S–O interaction in the thiazole nucleosides. Ara-tiazofurin (2-β-D-arabinofuranosylthiazole-4-carboxamide, ara-T) is the inactive arabinose analogue of tiazofurin. The crystal structure of ara-T is reported. This structure provides evidence for an attractive S–O interaction not seen in the other thiazole nucleosides. The conformation about the *C*-glycosyl bond in ara-T is such that close contacts are formed between the thiazole sulfur and both O1′ *and* the 2′-hydroxyl oxygen O2′. This conformation is interpreted in terms of an additional attractive interaction between S and O2′. This interpretation is supported by comparison of the conformation of ara-T with those of other ara-nucleosides. These findings provide further evidence for an attractive S–O interaction in the thiazole nucleosides. Ara-T also demonstrates a second conformational feature found in these compounds: the carboxamide nitrogen remains cis to the thiazole nitrogen. Implications of these potentially constrained conformational features are discussed in terms of the mechanism of activity of tiazofurin.

Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide, NSC 286193, Figure 1a) is a novel *C*-glycosyl thiazole that has demonstrated significant antitumor activity in a number of model tumor systems. Tiazofurin is curative in vivo for the Lewis lung carcinoma in mice[1] and has demonstrated in vitro activity against both human lymphoid[2] and lung tumor[3] cell lines and in vivo activity

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