Synthesis and DNA Binding Properties of γ-Carbolinium Derivatives and Benzologues

Andrés Molina,† Juan J. Vaquero,† José L. Garcia-Navio,† Julio Alvarez-Builla,*,†
Beatriz de Pascual-Teresa,‡ Federico Gago,‡ María M. Rodrigo,§ and Milagros Ballesteros⊥

Departamento de Química Orgánica, Departamento de Farmacología, Departamento de Química-Física, and Departamento de Química Analítica, Universidad de Alcalá de Henares, 28871-Alcalá de Henares, Madrid, Spain

Received February 8, 1996⊗

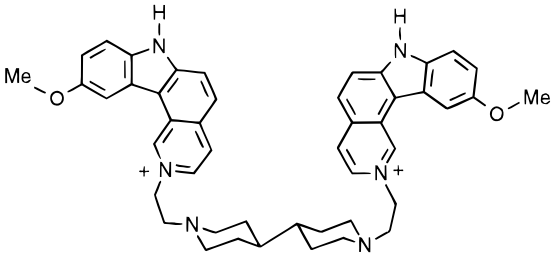
The 5*H*-pyrido[4,3-*b*]indole, 11*H*-indolo[3,2-*c*]quinoline, 5*H*-benzo[*f*]pyrido[4,3-*b*]indole, and 13*H*-benz[5,6]indolo[3,2-*c*]quinoline heteroaromatic nuclei have been synthesized by the Graebe−Ullmann method by classical heating or under microwave irradiation. These tri-, tetra-, and pentacyclic compounds were transformed into the corresponding cationic derivatives by N-alkylation, and the DNA-binding properties of the resulting cationic systems were examined using UV−vis spectroscopy, viscometric determinations, and molecular modeling techniques. The tetracyclic cations were transformed into bis-salts by means of a diethyl bispiperidine rigid chain and a more flexible polyamide linker, but the low solubility of these bis-salts made the study of their bisintercalating properties difficult.

Introduction

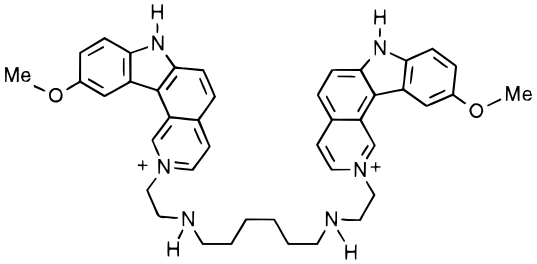
The intercalation[1] of planar aromatic molecules into the DNA double helix results in dramatic changes in DNA conformation[2] and can inhibit DNA replication, transcription, and/or topoisomerase activities and is considered to be crucial in the medicinal action of some antimytotic drugs.[3]

In recent years the search for new and better anticancer drugs has led to the preparation of bisintercalators with greater DNA affinities by oligomerization of DNA intercalators.[4] Ditercalinium (**1**)[5] is one such prototypical agent (Chart 1) and gives rise to cell death by malfunction of the DNA repair system[6] associated with unstacking and bending of DNA as observed in the reported ditercalinium−[d(CGCG)]$_2$ complex.[7] It was not expected that DNA complex formation with analogs having a more flexible linker would provoke these DNA modifications. However, the recent findings where the same DNA sequence complexed with Flexi-Di (**2**)[5c,d] was found to be bent to a degree exceeding that of the ditercalinium complex[8] indicated that rigidity of the linker was not essential to increasing the bending in these complexes.

Chart 1



1



2

Although earlier studies with ditercalinium and Flexi-Di have proved, as have recent reports on the importance of the electrostatic term in the stacking interactions,[9] that a polycyclic planar aromatic cation is essentially required for strong DNA intercalation, it remains unclear how to optimize this interaction. Thus, the synthesis of new bisintercalators with structures differing in length, rigidity, and functionality of the

† Departamento de Química Orgánica.
‡ Departamento de Farmacología.
§ Departamento de Química-Física.
⊥ Departamento de Analítica.
⊗ Abstract published in *Advance ACS Abstracts,* July 15, 1996.

(1) For pioneering works on intercalation, see: (a) Lerman, L. S. *J. Mol. Biol*. **1961**, *3*, 18. (b) Lerman, L. S. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *49*, 94.

(2) Wilson, W. D.; Jones, R. In *Intercalation Chemistry*; Whittingham, M. S., Jacobson, A. J., Eds.; Academic Press: New York, 1981; Chapter 14.

(3) Denny, W. A.; Baguley, B. C.; Cain, B. F.; Waring, M. J. In *Mechanism of Action of Anticancer Drugs*; Neidle, S., Waring, M. J., Eds.; MacMillan: London, 1983.

(4) For reviews, see: Wakelin, L. P. G. *Med. Chem. Rev.* **1986**, *6*, 275.

(5) (a) Roques, B. P.; Le Pecq, J. B.; Pelaprat, D.; Le Guen, I. French Patent *78.23801,* 1978. (b) Roques, B. P.; Pelaprat, D.; Le Guen, I.; Porcher, G.; Gosse, C.; Le Pecq, J. B. *Biochem. Pharm*. **1979**, *28*, 1811. (c) Pelaprat, D.; Oberlin, R.; LeGuen, I.; Roques, B. P.; Le Pecq, J. B. *J. Med. Chem*. **1980**, *23*, 1330. (d) Pelaprat, D.; Delbarre, A.; Oberlin, R.; LeGuen, I.; Roques, B. P.; Le Pecq, J. B. *J. Med. Chem*. **1980**, *23*, 1336. (e) Delbarre, A.; Delapierre, M.; Garbay, C.; Igolen, J.; Le Pecq, J. B.; Roques, B. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2155.

(6) (a) Lambert, B.; Segal-Bendirdjian, E.; Esnault, C.; Le Pecq, J. B.; Roques, B. P.; Jones, B.; Yeung, A. T. *Anticancer Drug Des*. **1990**, *5*, 43. (b) Lambert, B.; Roques, B. P.; Le Pecq, J. B. *Nucleic Acid Res.* **1988**, *16*, 1063.

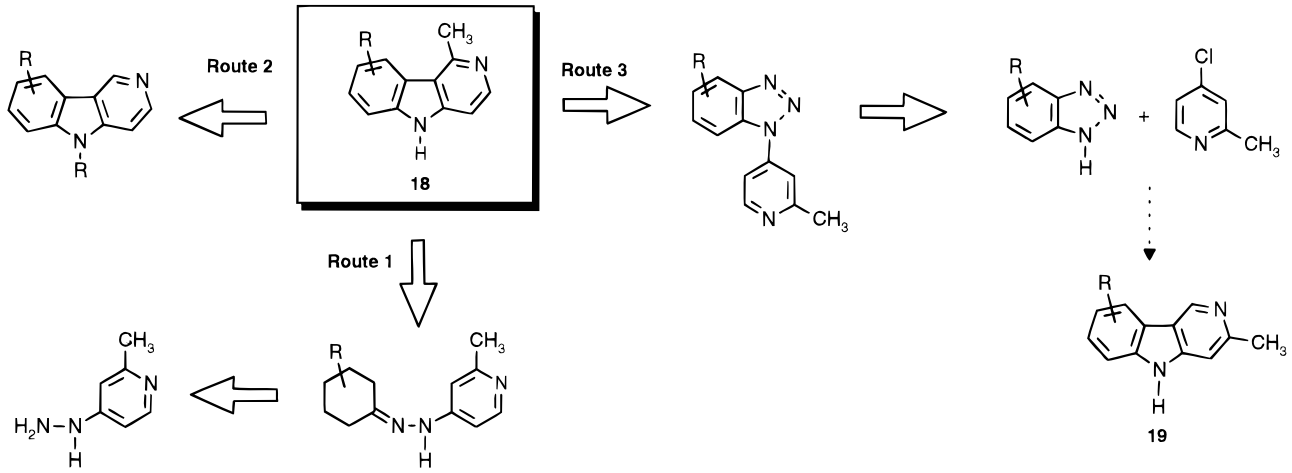
(7) Gao, Q.; Williams, L. D.; Egli, M.; Rabinovich, D.; Le Chen, S.; Qugley, G.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2422.

(8) Peek, M. E.; Lipscomb, L. A.; Betrand, J. A.; Gao, Q.; Roques, B. P.; Garbay-Jaureguiberry, C.; Williams, L. D. *Biochemistry* **1994**, *33*, 3794.

(9) (a) Gallego, J.; Ortiz, A. R.; Gago, F. *J. Med. Chem*. **1993**, *36*, 1548. (b) Gallego, J.; Luque, F. J.; Orozco, M.; Burgos, C.; Alvarez-Builla, J.; Rodrigo, M. M.; Gago, F. *J. Med. Chem*. **1994**, *37*, 1602. (c) Gallego, J.; Luque, F. J.; Orozco, M.; Gago, F. *J. Biomol. Str. Dyn*. **1994**, *12*, 111.

S0022-3263(96)00266-6 CCC: $12.00

Chart 2



linking chain and in the intercalating chromophore itself could lead to an improved understanding of the interaction of intercalators with DNA and to the preparation of improved chemotherapeutic agents.

The above considerations stimulated our interest in different heterocycles as candidates for transformation into polycyclic cations with DNA-binding properties. One of these heteroaromatic systems was that based on γ-carboline and its benzo derivatives. From these systems we built up two types of heteroaromatic cations, one of them having a quaternary bridgehead nitrogen atom and the other one being an *N*-alkylazinium derivative. The synthesis of various derivatives of the latter, their transformation into bis-salts, and their DNA-binding properties, including a molecular modeling study, are reported herein.

Computational Methods

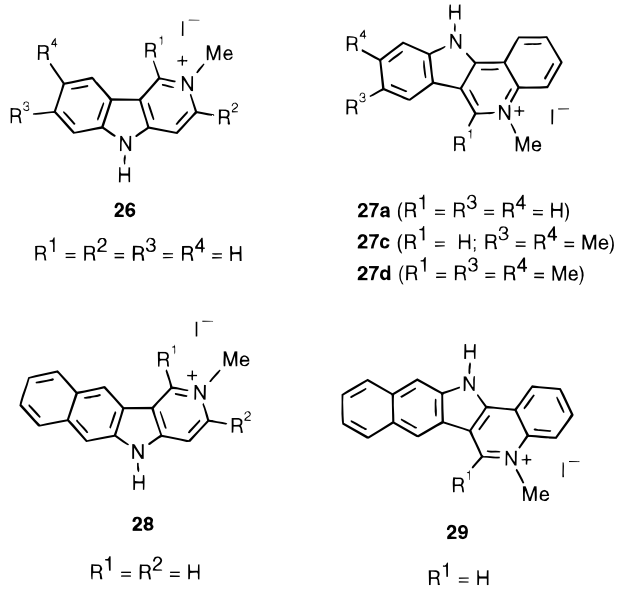
The cationic systems **27** were model-built with the Insight[10] program using standard bond lengths and angles, and geometries were fully optimized by means of the *ab initio* quantum mechanical program Gaussian 92[11] using the STO-3G basis set. Atom-centered point charges for the optimized structures were derived[12] which best reproduced the electrostatic potential of the molecules calculated by means of a single-point calculation using the larger 6-31G* basis set. The AMBER[13] all-atom force field parameters[14] were used for the DNA dimer, and covalent and nonbonded parameters for the intercalating chromophores of the ligands were derived by analogy or through interpolation[15] from those already present in the AMBER database.

The complex of ellipticine with 5-iodocytidylyl-(3′-5′)-guanosine[16] was retrieved from the Cambridge Structural Database (ref EICGUA) and used as a template for modeling the DNA intercalation site at a CpG step. Models were constructed for the four possible orientations of each ligand relative to the DNA base pairs. Energy refinement was carried out in a continuum medium of relative permitivity $\epsilon = 4r_{ij}$ for simulating the solvent environment using 5000 steps of steepest descent energy minimization. In order to sample a larger extent of the conformational space, the two lowest energy complexes of **27a** were subjected to molecular dynamics simulations at 300 K for 100 ps. To this end, a harmonic potential with a force constant of 10 kcal mol^{-1} Å^{-2} was used to restrain the atoms of the phosphate−sugar backbone to their positions at 0 ps, and the G:C hydrogen bonds were reinforced by means of an upper-bound harmonic restraining function with a force constant of 5 kcal mol^{-1} Å^{-2}.

Results and Discussion

Synthesis of DNA Intercalators and Bis-Salts. Since 1-methyl- or 3-methyl-γ-carboline derivatives are the substrates for building up polycyclic cations with a quaternary bridgehead nitrogen and the presence of methyl substituents on the pyrido[4,3-*b*]carbazole series has been associated with enhancement of DNA affinity and antineoplastic activity,[5c,17] the preparation of 1-methyl-5*H*-pyrido[4,3-*b*]indole derivatives **18** was the initial target (Chart 2). Our first approach to this series was a Fischer reaction[18] of an appropriately substituted cyclo-

Chart 3



(10) Insight II, version 2.2.0 (1994). Biosym Technologies Inc., 9685 Scranton Road, San Diego, CA 92121-2777.

(11) Gaussian 92, Revision D.2. Frisch, M. J.; Trucks, G. W.; Head-Gordon, M.; Gill, P. M. W.; Wong, M. W.; Foresman, J. B.; Johnson, G.; Schlegel, H. B.; Robb, M. A.; Replogle, E. S.; Gomperts, R.; Andres, J. L.; Raghavachari, K.; Binkley, J. S.; González, C.; Martin, R. L.; Fox, D. J.; Defrees, D. J.; Baker, J.; Stewart, J. J. P.; Pople, J. A.; Gaussian, Inc., Pittsburgh, PA, 1992.

(12) Singh, U. C.; Kollman, P. A. *J. Comput. Chem.* **1984**, *5*, 129.

(13) Pearlman, D. A.; Case, D. A.; Caldwell, J.; Seibel, G.; Singh, U. C.; Weiner, P.; Kollman, P. A. AMBER version 4.0, 1991. Department of Pharmaceutical Chemistry, University of California, San Francisco.

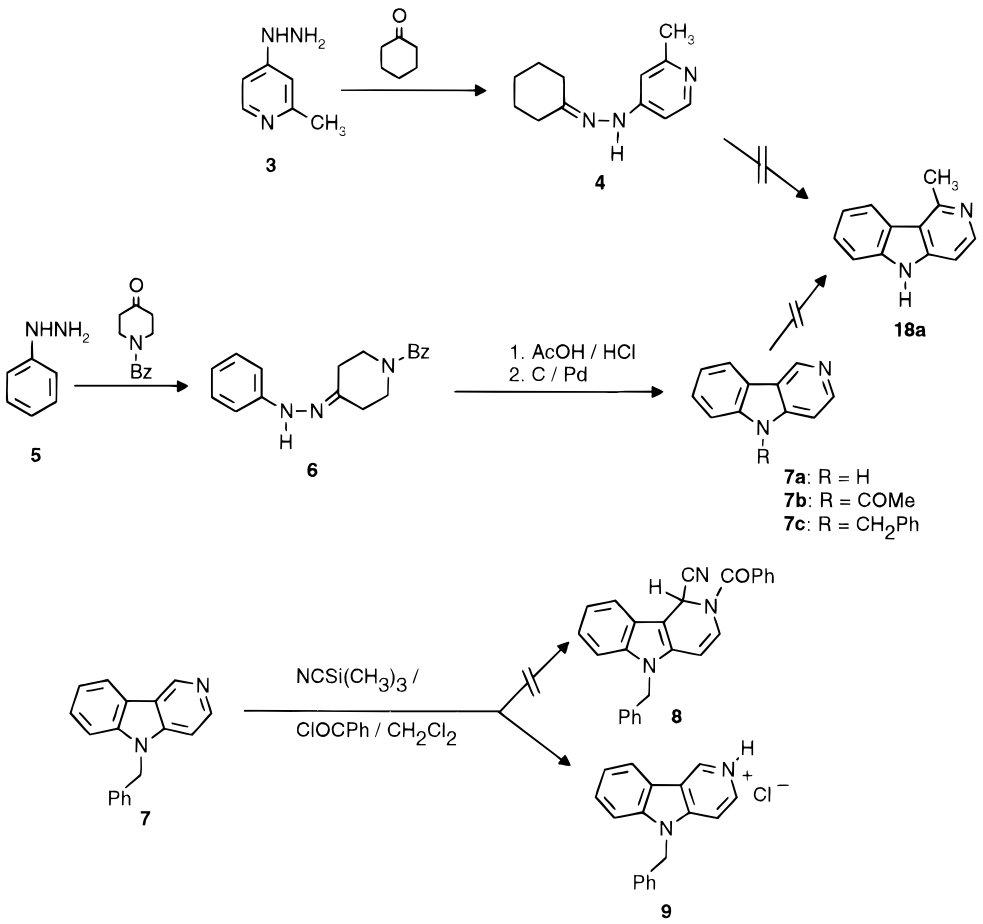
(14) Weiner, S. J.; Kollman, P. A.; Nguyen, D. T.; Case, D. A. *J. Comput. Chem.* **1986**, *7*, 230.

(15) Weiner, S. J.; Kollman, P. A.; Case, D. A.; Singh, U. C.; Ghio, C.; Alagona, G.; Profeta, S.; Weiner, P. *J. Am. Chem. Soc.* **1984**, *106*, 765.

(16) Jain, S. C.; Bhandary, K. K.; Sobell. H. M. *J. Mol. Biol.* **1979**, *135*, 813.

(17) Le Pecq, J. B.; Xuang, N. D.; Gosse, C.; Paoletti, C. *Proc. Nat. Acad. Sci. U.S.A.* **1974**, *71*, 5082.

Scheme 1



hexanone with a 4-hydrazinopyridine incorporating the methyl substituent[19] (route 1, Chart 2). This approach, however, was abandoned after several vain attempts in neutral media (diethylene glycol, reflux) or using different acids such as HCl/AcOH, H_2SO_4, or $ZnCl_2$. The lack of success was probably due to the low activation of the pyridine ring in **4** toward electrophilic attack (Scheme 1).

The second approach attempted to convert the γ-carboline to **18** by direct methylation of its protected precursor (route 2, Chart 2). The intermediate **7** can be obtained by the Fischer reaction[20] from **6** which in turn is obtained by condensation of phenylhydrazine **5** and benzylpiperidone. Attempts to react **7b** (R = COMe) and **7c** (R = CH_2Ph) with dimethyl sulfoxide in the presence of sodium hydride and ultrasound[21] yielded **7a** in the first case and the unaltered starting material in the latter. Attempts to obtain **18** via the Reissert compounds **8** also failed as **8** could not be generated with alkaline cyanides[22] or trimethylsilyl cyanide,[23] the salt **9** being isolated as the main reaction product in the latter case (Scheme 1).

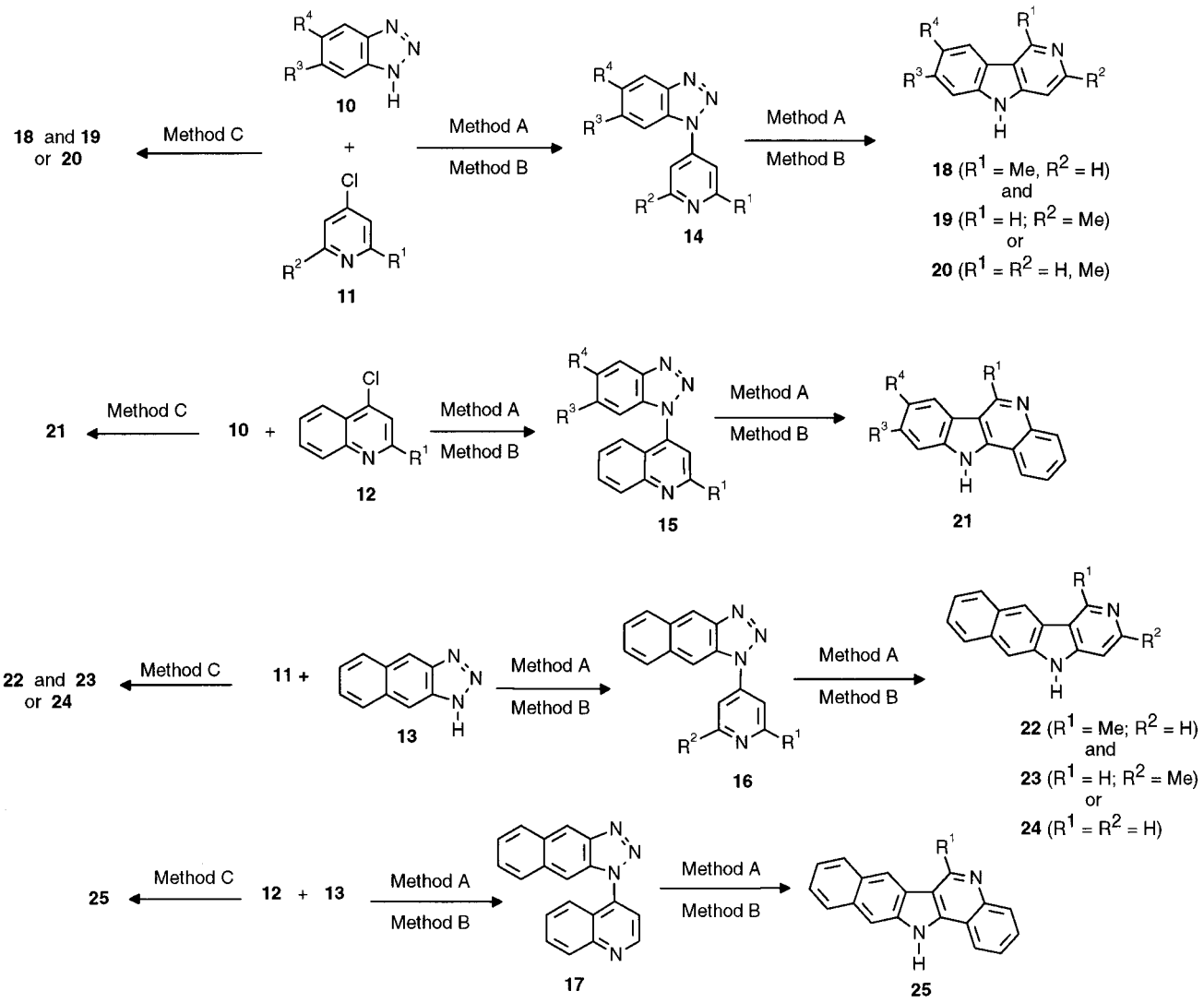
We therefore turned our attention to the Graebe–Ullmann[24] reaction which entailed the preparation of a 4-pyridylbenzotriazole derivative which upon treatment with acid gave the fully aromatic γ-carboline through nitrogen extrusion.[25] Against this strategy was the possible formation of both regioisomers, 1-methyl-γ-carboline (**18**) and 3-methyl-γ-carboline (**19**), in the thermolysis of the pyridylbenzotriazole (route 3, Chart 2). Although the formation of both isomers was confirmed to occur (Scheme 2), fortunately, chromatography allowed them to be separated and both could be employed as substrates for the preparation of azonia derivatives with bridgehead nitrogens,[26] the results of which will be reported elsewhere.

Although this third approach was much more successful and simple, the preparation of the pyridylbenzotriazole by condensation of the 4-chloroazine derivative and the corresponding benzotriazole required high temperatures (150 °C) with even higher temperatures (150−200 °C) sometimes being needed in the subsequent thermolysis (Scheme 2, method A). The yields of γ-carboline varied greatly depending on the temperature control, especially in the thermolysis step.[25]

To overcome this problem we focused on the development of a more efficient and simpler Graebe–Ullmann

(18) (a) Robinson, B. *Chem. Rev.* **1969**, *69*, 227. (b) Robinson, B. *The Fischer Indole Synthesis*; Wiley: New York, 1983.
(19) Crooks, P. A.; Robinson, B. *Can. J. Chem.* **1969**, *47*, 2061.
(20) Buu-Höi, N. P.; Roussel, O.; Jacquignon, P. *J. Chem. Soc.* **1964**, 708.
(21) Ezquerra, J.; Alvarez-Builla, J. *Org. Prep. Proc. Int.* **1985**, *17*, 190.
(22) Popp, F. D. *Avd. Heterocycl. Chem.* **1968**, *9*, 1.
(23) Ruchirawat, S.; Phadungkul, N.; Chuankamnerdkan, Thebtaranonth, C. *Heterocycles* **1977**, 6.
(24) Graebe, C.; Ullman, F. *Justus Liebigs Ann. Chem.* **1896**, *291*, 16.
(25) For examples, see: (a) Laurson, W.; Perkin, W. H.; Robinson, R. *J. Chem. Soc.* **1924**, *125*, 626. (b) Freak, R. H.; Robinson, R. *J. Chem. Soc.* **1938,** 2013. (c) Kaczmarek, L.;. Balicki, R.; Nantka-Namirski, P. *Arch. Pharm.* **1988**, *321*, 463. (c) Parrick, J.; Yahya, J. *J. Chem. Res., Synop. 1, J. Chem. Res., Miniprint* **1990**, 201. (d) Mehta, L. K.; Parrick, J.; Payne, F. *J. Chem. Soc., Perkin Trans 1* **1993**, 1261.
(26) For recent examples, see: (a) Santiesteban, I.; Siro, J. G.; Vaquero, J. J.; García-Navio, J. L.; Alvarez-Builla, J.; Castaño, O.; Andrés, J. L. *J. Org. Chem.* **1995**, *60*, 5667. (b) Diaz, A.; Matia, M. P.; García-Navío, J. L.; Vaquero, J. J.; Alvarez-Builla, J. *J. Org. Chem.* **1994**, *59*, 8294.

Scheme 2[a]



[a] **14**−**17**: method A, 150−200 °C (30 min); method B, MW (160 W, 7−10 min). **18**−**25**: method A, (a) 150−200 °C (30 min); (b) isolation of the pyridylbenzotriazole; (c) $H_4P_2O_7$, 150−210 °C (1−2 h); method B, (a) MW (160 W, 7−10 min); (b) isolation of the pyridylbenzotriazole; (c) $H_4P_2O_7$, MW (160 W, 4−6 min); method C, MW (160 W, 7−10 min) then $H_4P_2O_7$, MW (160 W, 4−6 min).

procedure for the synthesis of the γ-carboline system and some of its benzo analogues. As a result we found that when an equimolar mixture of 4-chloropyridine and 1*H*-benzotriazole was placed in conventional glassware and irradiated with microwaves, the condensation product **14c** was obtained in moderate yield, which however could be boosted to 88% by optimizing the reaction conditions (power and reaction time).[27,28] Subsequent microwave irradiation of the isolated pyridylbenzotriazole in the presence of pyrophosphoric acid gave the desired γ-carboline **20a** (Scheme 2, method B) with attendant evolution of nitrogen. Interestingly, when the crude reaction mixture containing the pyridylbenzotriazole **14c** was irradiated in the presence of the acid, the expected **20a** was isolated with no significant diminution of yield (Scheme 2, method C). This result was convenient since it eliminated the need to isolate the pyridylbenzotriazole derivative **14c**. Using similar microwave conditions to form **14d**−**f**, the subsequent microwave-induced nitrogen extrusion also gave the derivatives of the 5*H*-pyrido[4,3-*b*]indole systems **20d**−**f** in a simple one-pot procedure in similar or higher yields than those from the classical method and in all cases with a reduction in reaction time from about 2 h to about 15 min (Scheme 2, method C). Analogously the microwave condensation of the benzotriazole derivatives **10** with the 4-chloroquinoline derivatives **12** and subsequent microwave decomposition afforded the 11*H*-indolo[3,2-*c*]quinoline derivatives **21**. The procedure was also successfully applied to the reaction of 1*H*-naphtho[2,3-*d*][1,2,3]triazole (**13**) and 4-chloro-2-picoline derivatives yielding a 1:1 separable mixture of the tetracyclic 5*H*-benzo[f]pyrido[4,3-*b*]indole derivatives **22** and **23** or compound **24** when the 4-chloropyridine itself was reacted with **13**. Finally, the pentacyclic 13*H*-benz[5,6]indolo[3,2-*c*]quinoline derivatives **25** were the products in the reaction of **13** with 4-chloroquinoline derivatives **12**.

The tri-, tetra-, and pentacyclic systems thus obtained were easily transformed into the cations **26**−**28** by simple N-alkylation with methyl iodide at room temperature.

(27) For a preliminary communication: Molina, A.; Vaquero, J. J.; Garcia-Navio, J. L.; Alvarez-Builla, J. *Tetrahedron Lett.* **1993**, *34*, 2673.

(28) For reviews on microwaves in organic synhesis, see: (a) Giguere, R. J. *Org. Synth.: Theory Appl.* **1989**, *1*, 103. (b) Mingos, M. P.; Baghurst, D. R. *Chem. Soc. Rev.* **1991**, *20*, 1. (c) Bram, G.; Loupy, A.; Villemin, D. In *Solid Supports and Catalysis in Organic Synthesis*; Smith, K., Ed.; Ellis Horwood: New York, 1992; pp 302. (d) Caddick, S. *Tetrahedron* **1995**, *51*, 10403.

Table 1. Pyridylbenzotriazole 14−17 and γ-Carbolines Derivatives 18−25 Prepared

						pyridylbenzotriazole[a]		γ-carboline derivative[b,c]	
entry	R^1	R^2	R^3	R^4	method	no.	yield (%)	no.	yield (%)
1	Me	H	H	H	A	**14a**	48	**18a**	28
2	H	Me	H	H	A	**14a**		**19a**	34
3	Me	H	Me	Me	A	**14b**	47	**18b**	42
4	H	Me	Me	Me	A	**14b**		**19b**	47
5	H	H	H	H	A	**14c**	89	**20a**	70
6					B	**14c** [7]	88	**20a** [6]	70
7					C	−	−	**20a** [7, 6]	60
8	H	H	Me	Me	A	**14d**	94	**20b**	77
9					B	**14d** [10]	93	**20b** [4]	78
10					C	−	−	**20b** [10, 4]	71
11	Me	Me	H	H	A	**14e**	40	**20c**	48
12	Me	Me	Me	Me	A	**14f**	44	**20d**	67
13	H	−	H	H	A	**15a**	96	**21a**	83
14					B	**15a** [7]	92	**21a** [7]	83
15					C	−	−	**21a** [7, 7]	76
16	Me	−	H	H	A	**15b**	81	**21b**	43
17					C	−	−	**21b** [7, 6]	32
18	H	−	Me	Me	A	**15c**	92	**21c**	85
19					C	**15c**	−	**21c** [10, 4]	81
20	Me	−	Me	Me	A	**15d**	83	**21d**	58
21					C	−	−	**21d** [10, 5]	48
22	Me	H	−	−	A	**16a**	30	**22**	38
23	H	Me	−	−	A	**16a**		**23**	41
24	H	H	−	−	A	**16b**	70	**24**	40
25					C	−	−	**24** [10,6]	30
26	H	−	−	−	A	**17a**	78	**25a**	64
27					C	−	−	**25a**[10,5]	45
28	Me	−	−	−	A	**17b**	96	**25b**	41
29					C	−	−	**25b**[10,5]	35

[a] Number in brackets in the pyridylbenzotriazole series refers to the optimized microwave irradiation time (min) with isolation of compounds **14**−**17** (method B). [b] Number in brackets in the γ-carboline series refers to method B and are the optimized microwave irradiation times (min) to achieve **20**−**25** from the corresponding isolated pyridylbenzotriazole derivatives. The first number in brackets in the γ-carboline series in method C refers to the formation of the benzotriazole derivative and the second to its transformation into γ-carboline derivatives. [c] Overall yields are given in method C.

Table 2. DNA Binding and Cytostatic Activity of Cationic Derivatives 26−29[a]

no.	λ_{free}	λ_{bound}	ϵ_{free}	ϵ_{bound}	λ_{IP}	$10^5 K$	n	m	cellular toxicity on HT-29 (ED_{50})
26	nm[b]	nm[b]	nm[b]	nm[b]	nm[b]	nm[b]	nm[b]	0.23	ns
27a	346	354	9323	5263	366	1.68	3.49	1.15	0.7
27c	354	364	9144	5131	374	1.43	3.11	1.18	0.6
27d	350	362	9800	6168	368	3.31	3.06	1.89	0.4
28	340	348	5547	5068	362	nm[c]	nm[c]	0.71	5.0
29	388	406	6113	4116	420	nm[c]	nm[c]	1.07	nd
EtBr						12.0	2.00	1.11	
Doxr									2.5

[a] K, affinity constant for DNA (M^{-1}). n, number of base pairs occluded by each bound ligand molecule. m, helix extension slope measured by sonicated DNA viscosimetric lengthening; values within 0.08−0.12 error. Cellular toxicity (ED_{50}) is the dose which inhibits cellular growth by a factor of 50% (in μM). EtBr, ethidium bromide. Doxr, doxorubicine. nd, not determined. [b] nm, not measured due to absorption maxima overlaping with DNA absorption or [c]due to low hypochromicity of the absorption maximum. ns, not significant. λ_{free} and λ_{bound}, wavelength of maximum absorption for free and bound to DNA compounds. ϵ_{free} and ϵ_{bound}, extinction coefficients for free and bound to DNA compounds. λ_{IP}, wavelength of isosbestic point.

However, the N-methylation of the pentacyclic derivative **21d** to give **29** required refluxing for 10 h.

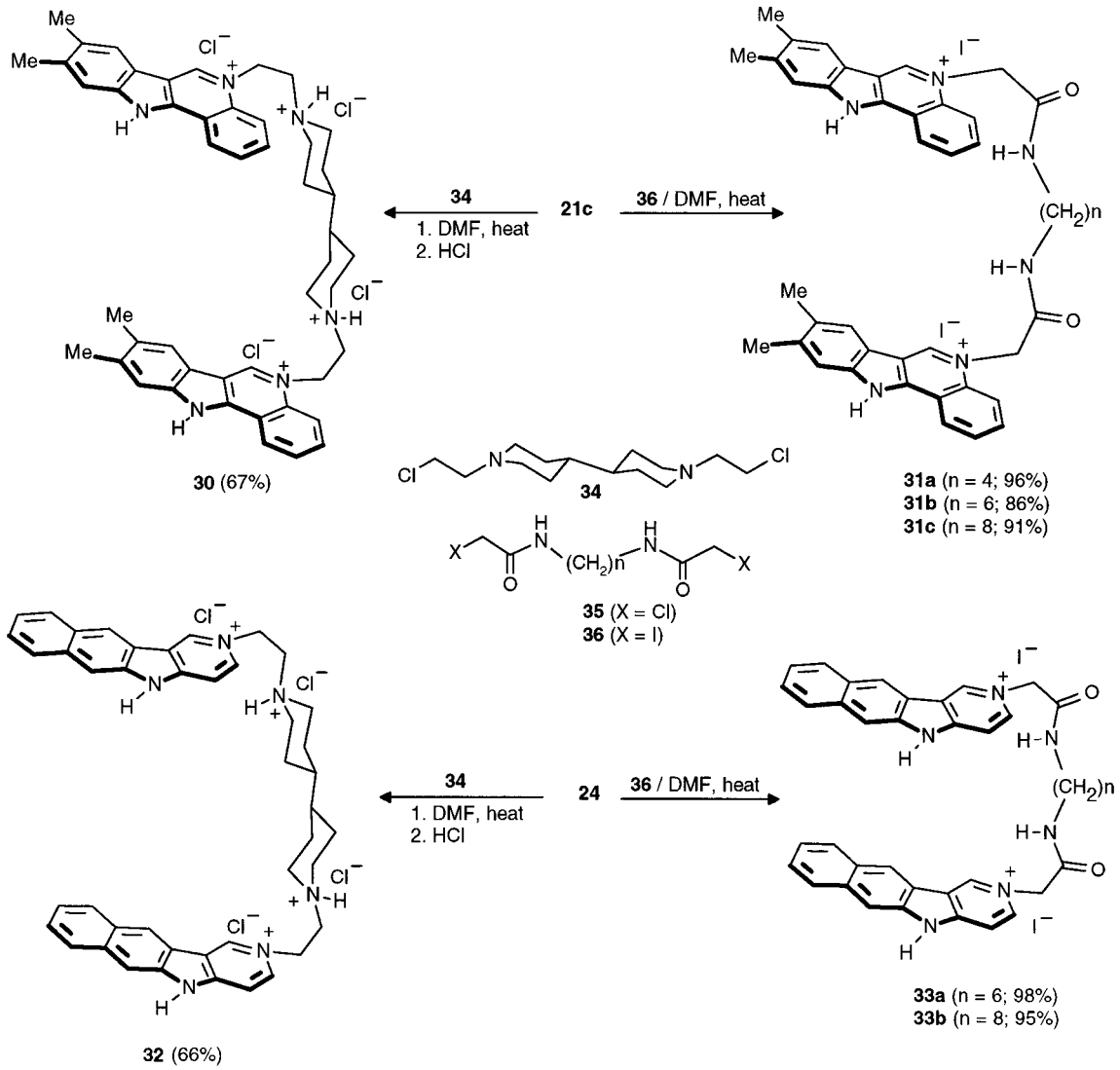
The lack of intercalating properties found for the cationic system **26** (see below in DNA binding studies) and the higher cytostatic activity against colon carcinoma HT-29 line cells found in series **27** (Table 2) led us to dimerize **21c** and **24** as models of nonlinear and linear tetracyclic systems, respectively. Thus the bis-salts **30** and **32** were obtained by quaternization of 2 equiv of the unprotected precursors **21c** and **24**, respectively, with 1 equiv of the 1,1′-bis(2-chloroethyl)-4,4′-bipiperidine (**34**) in hot *N*,*N*-dimethylformamide (DMF). Analogously, in the more flexible dimers **31** and **33** the diamide linker was connected to the chromophores by N-alkylation with the corresponding diiodo derivative **36** under the same conditions (Scheme 3).

Unfortunately all the bis-salts **30**−**33** obtained were insufficiently soluble in various buffers for reliable data on binding interactions to be obtained. Moreover, when the cytotoxicity of the bis-salts **30** ($ED_{50} = 2 \times 10^{-6}$) and **31** ($ED_{50} = 1 \times 10^{-5}$ to 1×10^{-6}) was compared with that of monocationic chromophores **27** (Table 2), a significant decrease in the activity was found, especially when a shorter flexible linker was introduced. Compound **33** ($ED_{50} > 10^{-5}$) was also lees active than **28**, while the more rigid chain in **32** ($ED_{50} = 7 \times 10^{-6}$) appears to only moderately reduce the activity.

DNA Binding Studies. Several techniques were used to evaluate the DNA intercalating properties[29] of com-

(29) For criteria for the mode of binding to DNA, see: Suh, D.; Chaires, J. B. *BioMed Chem.* **1995**, *3*, 723. (b) Long, E. C.; Barton, J. K. *Acc. Chem. Res.* **1990**, *23*, 2773.

Scheme 3



pounds **26**−**29**. In some cases it was not possible to obtain data for a compound due to low solubility, while in other cases affinity constants could not be determined from the absorption spectra due to band overlapping. Whereas addition of compounds **18**−**25** to a sample of calf thymus DNA in Tris·HCl buffer ([NaCl] = 0.05 M, pH = 7.5) induced hyperchromicity, probably due to dipole-dipole interactions, the corresponding *N*-alkyl derivatives **27**−**29** gave hypochromic and bathochromic shifts on complex formation.[30] With the *N*-alkyl derivative **26** it was not possible to obtain data because this compound has an absorption maximum in the DNA absorption range. The hypochromicities in the absorption maxima for **28** and **29**, although well removed from the DNA absorption maxima, were low and thus greatly hindered accurate determination of their DNA-binding constants. At pH = 7.5 in Tris buffer containing 15 mmol L^{-1} NaCl, the maximum hypochromicity percent found was 43% for **27c**, whose absorption behavior is shown in Figure 1. An isosbestic point appears, indicating that these compounds present a unique binding mode with DNA.[30] From the spectral changes, nonlinear Scatchard binding isotherms were generated for the interaction of these compounds with DNA (Figure 2). The interpretation of such data depends on the theoretical model used. For practical purposes we have found the treatment reported by McGhee and Von Hippel[31] to be the most convenient. This treatment has shown that a curved

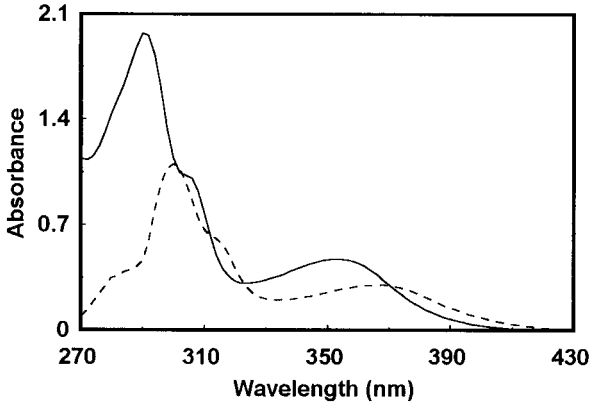


Figure 1. Effect of calf thymus DNA on the ultraviolet absorption spectrum of **27c** in Tris·HCl (pH = 7.5). The solid line indicates the spectrum of **27c** alone at a concentration of 5.25×10^{-6} M, and the dashed line represents the spectrum of **27c** at the same concentration in the presence of 1.80×10^{-4} M DNA (nucleotide molarity).

(30) Binding commonly results in hypochromism and a shift of the transition to longer wavelength in the intercalated chromophore. (a) Dougherty, G.; Pigram, W. J *CRC Crit. Rev. Biochem.* **1982**, *12*, 103. (b) Berman, H. M.; Young, P. R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 87.

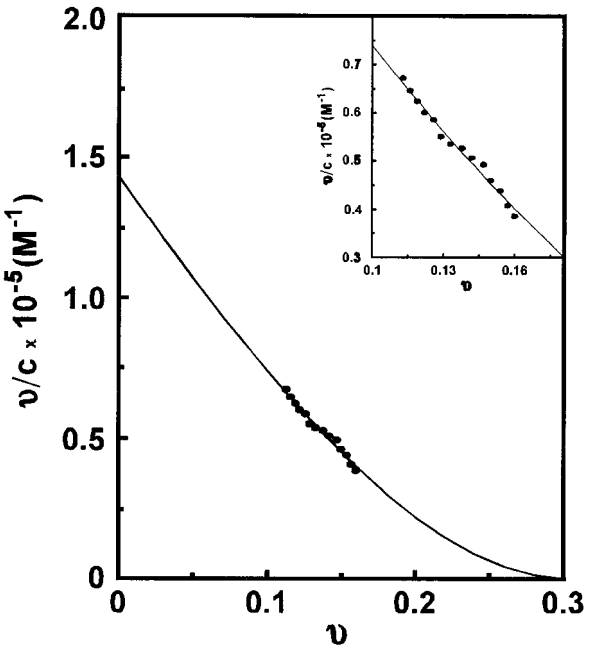


Figure 2. Scatchard plot for the binding of **27c** to calf thymus DNA. Values for the ligand binding ratio v (molar ratio of bound compound per DNA nucleotide) and free compound concentration c were determined from data taken from spectrophotometric titrations of the compound with DNA. Points in the figure correspond to the experimental data and solid lines to the best fits from the McGhee−von Hippel model.[31]

Scatchard plot is inevitable if the bound molecule occupies more than one base pair on the DNA. The points in Figure 2 are experimental values, and the solid lines result from those determined by a nonlinear least-squares computer program using the extended McGhee−Von Hippel equation[31] (see Experimental Section) where v is the ligand binding ratio and c is the free compound concentration. The binding parameters (K, n) determined in this manner are shown in Table 2 with K being the binding equilibrium constant and n the number of base pairs occluded per bound ligand molecule. All three derivatives have a DNA-binding site which spans 3−4 base pairs with affinity constant values around 10^5 M^{-1}, which although lower than the value for the well-known intercalator ethidium bromide,[32] used here as reference, are within the range of other binding-DNA intercalating agents such as chloroquine.[33] Dimethyl or trimethyl substitution on the chromophore had litttle effect on DNA-binding constants (for discussion see Molecular Modeling). As expected, the greater DNA affinity corresponded to the trimethyl derivative.

Unwinding and extension of the DNA backbone in intercalated complexes results in length and viscosity increases in sonicated DNA. When the viscosity of sonicated calf thymus DNA was measured in the absence and presence of increasing concentrations of the cationic compounds **26**−**29**, a plot of the relative increase in contour length L/L_0 versus r (Figure 3), where r is the molar ratio of added compound to DNA nucleotides,[4,34]

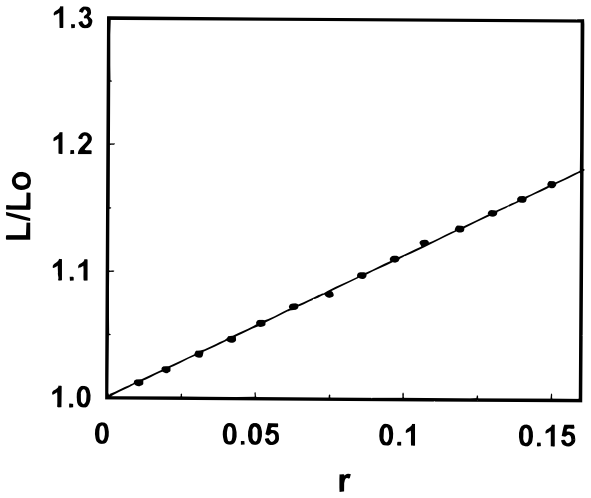


Figure 3. Relative length increase L/L_0 of **27c**−DNA complexes as a function of the molar ratio of added compound to DNA nucleotides r. The contour lengths in the presence (L) or absence (L_0) of the compound were calculated from viscosity measurements on sonicated calf thymus DNA.

gives a slope m, the value of which is shown for each compound in Table 2. Adopting the criterion of Cory[32,35] based on the pioneering methodology from Cohen and Eisenberg[34] for monofunctional intercalators such as ethidium bromide, proflavine, and aminoacridines which usually have values of m between 0.8 and 1.5, all the tetracyclic compounds **27** and the pentacyclic **29** have m values which fall within the range for intercalators while the tricyclic system **26** does not intercalate into DNA and thus may account for its lack of cytotoxicity. For the linear fused tetracyclic system **28**, its DNA intercalating ability is not clear on the basis of its DNA viscometric lengthening slope which is lower than that for **27** or **29**. Although this cationic compound retains a significant cellular toxicity when compared with series **27**, the aforementioned difficulties in measuring its binding affinity together with the extremely low solubility of its dimers precluded a wider study of its intercalative behavior and we are thus unable to confirm at this stage its intercalative aptitude.

Molecular Modeling. *Ab initio* molecular orbital calculations were performed in order to study the molecular charge distribution in these compounds. In addition, molecular modeling studies were undertaken to gain insight into the mode of intercalation at a 5′-CpG-3′ step. Simple monointercalators often show a preference for binding to G:C over A:T base pairs,[36] in accord with the larger surface area and the greater polarizability of the former base pair. In fact, the CpG sequence has been widely employed in crystallography to demonstrate intercalation,[37] and theoretical calculations on stacked base pairs show that CpG yields the weakest stacking interaction among other steps involving G:C pairs,[38] which favors formation of the intercalation site.

One important aspect of stacking interactions involving DNA bases is the strong influence that both composition

(31) McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469.
(32) (a) Cory, M.; Tidwell, R. R.; Fairley, T. A. *J. Med. Chem.* **1992**, *35*, 431. (b) Fairley, T. A.; Tidwell, R. R.; Donkor, I.; Naiman, N. A.; Ohemeng, K. A.; Lombardy, R. J.; Bentley, J. A.; Cory, M. *J. Med. Chem.* **1993**, *36*, 1746. (c) Graves, D. E.; Watkins, C. L.; Yielding, L. W. *Biochemistry* **1981**, *20*, 1887.
(33) (a) Cohen, S. N.; Yielding, K. L. *J. Biol. Chem.* **1965**, *240*, 3123. (b) Feignon, J. Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450.
(34) (a) Cohen, G.; Eisenberg, H. *Biopolymers* **1966**, *4*, 429. (b) Cohen, G.; Eisenberg, H. *Biopolymers* **1969**, *8*, 45.
(35) Cory, M.; Mckee, D. D.; Kagan, J.; Henry, D. W.; Miller, J. A. *J. Am. Chem. Soc.* **1985**, *107*, 2528.
(36) Müller, W.; Crothers, D. M. *Eur. J. Biochem.* **1975**, *54*, 267.
(37) Neidle, S.; Pearl, L. H.; Skelly, J. V. *Biochem. J.* **1987**, *243*, 1.
(38) (a) Ornstein, R. L.; Rein, R.; Breen, D. L.; MacElroy, R. D. *Biopolymers* **1978**, *17*, 2341. (b) Aida, M.; Nagata, C. *Int. J. Quantum Chem.* **1986**, *29*, 1253.

Table 3. Partial Atomic Charges[a] for 27a, 27c and 27d

27a		27c		27d	
C1	−0.176 660	C1	−0.210 816	C1	−0.198 145
H1	0.167 551	H1	0.192 760	H1	0.167 184
C2	−0.121 617	C2	−0.162 287	C2	−0.101 136
H2	0.168 318	H2	0.194 732	H2	0.163 251
C3	−0.041 687	C3	−0.048 524	C3	−0.088 349
H3	0.165 101	H3	0.185 092	H3	0.171 359
C4	−0.263 906	C4	−0.328 223	C4	−0.211 056
H4	0.181 498	H4	0.211 078	H4	0.164 261
C4a	0.223 655	C4a	0.270 267	C4a	0.194 503
N5	−0.079 546	N5	−0.129 212	N5	−0.196 420
Me5	0.203 849	Me5	0.220 345	Me5	0.220 307
C6	0.009 309	C6	0.007 906	C6	0.383 540
H6	0.218 228	H6	0.230 325	Me6	0.070 515
C6a	−0.083 541	C6a	0.008 164	C6a	−0.202 729
C6b	−0.010 002	C6b	−0.090 976	C6b	0.104 996
C7	−0.206 116	C7	−0.290 560	C7	−0.418 113
H7	0.183 335	H7	0.225 407	H7	0.219 853
C8	−0.162 670	C8	0.069 395	C8	0.200 039
H8	0.174 019	Me8	0.015 473	Me8	−0.030 473
C9	−0.075 898	C9	0.216 046	C9	0.205 272
H9	0.171 903	Me9	0.008 988	Me9	−0.011 620
C10	−0.340 019	C10	−0.457 633	C10	−0.444 329
H10	0.211 573	H10	0.251 801	H10	0.238 327
C10a	0.350 612	C11	0.245 411	C10a	0.211 881
N11	−0.533 993	N11	−0.357 520	N11	−0.380 756
H11	0.415 993	H11	0.388 691	H11	0.381 168
C11a	0.223 153	C11a	0.061 335	C11a	0.117 638
C11b	0.027 558	C11b	0.072 535	C11b	0.069 033

[a] IUPAC numbering system.

and relative orientation of the stacked systems can have on overall stability.[39] This is due not only to variations in the dispersion and hydrophobic terms brought about by different extents of base overlap but also to the directionality dependence of the electrostatic contribution.[40] When accounting for DNA conformational preferences in echinomycin−DNA complexes[10a,c] or for sequence-specific binding of echinomycin-like antibiotics,[10b] a predominant role for the electrostatic term has been suggested. In this regard, the charge distribution of a given chromophore might largely determine its relative orientation within the intercalation site and, at the same time, increase or decrease its binding affinity. This reasoning is particularly important in the design of bisintercalators since knowledge of this preferred orientation can be crucial for locating the spacer in either the minor[41] or the major groove.[9] Therefore, introduction of suitable substituents on a chromophore can be expected to modulate these preferences.

The effect of methylation on the charge distribution of **27a** and **27c,d** is reflected in Table 3. The molecular electrostatic potential of these chromophores (data not shown) displays the least positive region on the π face of the indole phenyl ring and the most positive region around the NH atoms.

The molecular mechanics calculations revealed DNA−ligand interaction energy differences among the four possible orientations explored for each complex, suggestive of preferred orientations for each chromophore within the intercalation site at the CpG step. From the resulting molecular dynamics trajectories, several low-energy complexes of **27a** were selected and further minimized. Neither the potential energy of the complexes nor the intermolecular interaction energies provided firm evidence that one complex was more stable than the other. The same can be said for the rest of the compounds docked in different orientations. There are many variables that this sort of study ignores, among them entropy contributions and solvation effects. Nevertheless, the results of this study provide some hints that methylation on key positions could have important effects both on the stability and the orientational preferences of the chromophores. These preferences would arise from both steric and electrostatic effects. Figure 4 shows the intercalated complexes.

In conclusion, this work presents a series of new 11*H*-indolo[3,2-*c*]quinolinium, 5*H*-benzo[*f*]pyrido[4,3-*b*]indolinium, and 13*H*-benz[5,6]indolo[3,2-*c*]quinolinium DNA intercalators. The presence of formal charges on these systems caused the expected batochromicity and hypocromicity in their UV spectra and the increase in viscosity in sonicated DNA. In addition, they displayed *in vitro* cytotoxicity in human carcinoma line cells. Disappointingly, dimer formation has little influence on cellular toxicity, and the low solubility of the dimers made it difficult to evaluate their potential behavior as bisintercalators. Support for the molecular modeling results can be provided by nuclear magnetic resonance techniques in order to understand the preferred orientation of these chromophores in the intercalative reaction. This work with synthetic oligonucleotides is now in progress, and results will be reported in due course.

Experimental Section

All melting points were measured in open capillary tubes and are uncorrected. IR spectral bands are reported in cm^{-1}. NMR spectra were recorded at 300 and 500 MHz, and the chemical shifts are expressed in parts per million (δ) relative to internal Me_4Si, with multiplicity, number of protons, and coupling constants in hertz. Microanalyses for all new compounds were performed in the analytical laboratory of the University. Mass spectra were obtained at an ionizing voltage of 70 eV. Absorbance measurements were made in the UV−vis region. Viscometric determinations were carried out on a Ubbelhode viscometer. A commercial microwave oven with 900 W of power output was used. Power generated by the oven was measured before every experiment by the method described by Wakins.[42] Molecular modeling studies were carried out in a Silicon-Graphics INDIGO workstation. Calf thymus DNA for viscosity and spectrophotometric binding analyses was purchased from the Sigma Chemical Co. The following compounds were prepared according to literature procedures: 5,6-dimethyl-1*H*-benzotriazole (**10b**),[43] 1*H*-naphtho[2,3-d][1,2,3]-triazole (**10c**),[44] 4-chloro-2-methylpyridine (**11b**),[45] 4-chloro-2,6-dimethylpyridine (**11c**),[45] (2-methylpyridin-4-yl)hydrazine hydrochoride (**3**),[46] 1,1′-bis(2-chloroethyl)-4,4′-bipiperidine dihydrochloride (**34**).[5]

***N*-Cyclohexylidene-*N*′-(2-methylpyridin-4-yl)hydrazine (4).** A mixture of 2-(methylpyridin-4-yl)hydrazine (**3**) (0.5 g, 3.14 mmol) and cyclohexanone (0.37 g, 37.5 mmol) in water (1 mL) was stirred at room temperatue overnight. It was then basified with a 20% solution of NaOH, extracted with Et_2O (3 × 8 mL), and dried over $MgSO_4$. The solvent was eliminated under reduced pressure to give an orange solid which was crystallized from MeOH-H_2O (0.45 g, 71%): mp 102−103 °C;

(39) Rein, R. In *Perspectives in Quantum Chemistry and Biochemistry*, Vol. II; Pullman, B., Ed.; John Wiley & Sons: New York, 1978; pp 307.
(40) (a) Bugg, C. E.; Thomas, J. M.; Sundaralingam, M.; Rao, S. T. *Biopolymers* **1971**, *10*, 175. (b) Hunter, C. A. *J. Mol. Biol.* **1993**, *230*, 1025.
(41) Spielmann, H. P.; Wemmer, D. E.; Jacobsen, J. P. *Biochemistry* **1995**, *34*, 8542
(42) Watkins, K. W. *J. Chem. Educ.* **1983**, *60*, 1043.
(43) Benson, F. R.; Hartzel, L. W.; Savell, W. L. *J. Am. Chem. Soc.* **1952**, *74*, 4917.
(44) Friedländer, P.; Zakrzewski, V. *Ber.* **1894**, *27*, 765.
(45) Talik, T.; Talik, Z. *Rocz. Chem.* **1968**, *42*, 2061.
(46) Itai, T.; Samiya, S. *Chem. Pharm. Bull.* **1961**, *9*, 87.

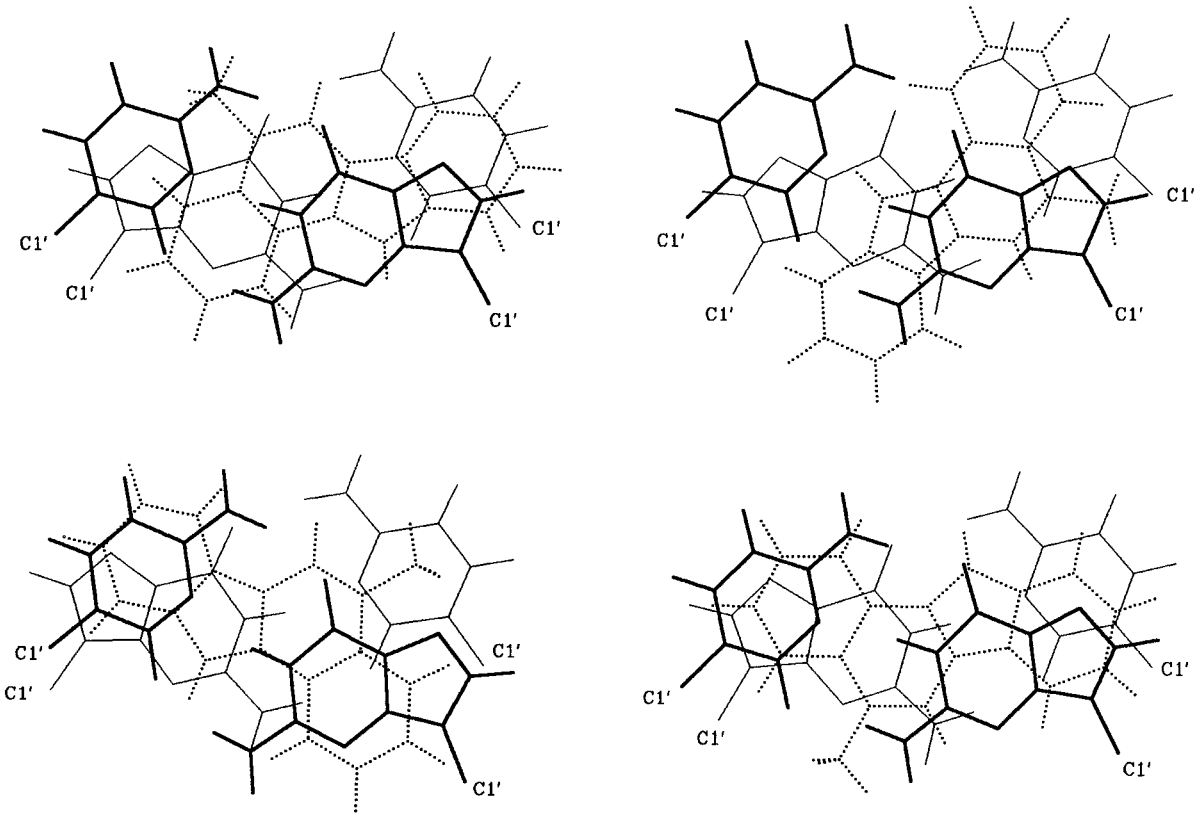


Figure 4. Complexes of **27a** with the CpG step after 100 ps of restrained molecular dynamics and energy minimization. Depending on the orientation of the chromophore relative to the intercalation site, different intermolecular interaction energies (ΔE_{inter}) and different intramolecular DNA energies (E_{DNA}) were obtained. Left to right, and top to bottom: $\Delta E_{inter} = -63.3, -58.9, -63.7, -65.8$ kcal mol^{-1}; $E_{DNA} = -144.1, -146.0, -141.8, -143.3$ kcal mol^{-1}.

IR (KBr) ν_{max} 3422, 2936, 1598, 1572, 1438, 1396, 1157 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 11.49 (bs, 1H), 8.58 (d, 1H, J = 5.0 Hz), 7.36 (s, 1H), 7.32 (d, 1H, J = 5.0 Hz), 2.5 (s, 3H), 1.69−1.45 (m, 10H) ppm. Anal. Calcd for $C_{12}H_{17}N_3$: C, 70.90; H, 8.43; N, 20.67. Found: C, 71.20; H, 8.35; N, 20.43.

1-Pyrido[4,3-*b*]indol-5-ylethanone (7b). A mixture of 5*H*-pyrido[4,3-*b*]indole (168 mg, 1 mmol), acetic anhydride (0.94 mL, 10 mmol), and pyridine (0.1 mL, 1.2 mmol) was stirred at room temperature for 30 min. The precipitate formed was filtered, dissolved in water, and extracted with CH_2Cl_2 (3 × 4 mL). The organic phase was dried over $MgSO_4$ and the solvent evaporated under reduced pressure to give a white solid which after recrystallization from EtOH gave 143 mg (68%) of white prisms: mp 98−99 °C; IR (KBr) ν_{max} 1707, 1365, 1304, 1284, 1191, 1011 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.35 (s, 1H), 8.66 (d, 1H, J = 6.0 Hz), 8.21−8.17 (m, 2H), 8.11 (d, 1H, J = 8.1 Hz), 7.58 (t, 1H, J = 8.1 Hz, J = 7.3 Hz), 7.48 (t, 1H, J = 7.7 Hz, J = 7.3 Hz), 2.92 (s, 3H) ppm. Anal. Calcd for $C_{13}H_{10}N_2O$: C, 74.26; H, 4.80; N, 13.33. Found: C, 74.45; H, 4.65; N, 13.50.

5-Benzyl-5*H*-pyrido[4,3-*b*]indole (7c). To a mixture of 5*H*-pyrido[4,3-*b*]indole (336 mg, 2 mmol), TBAB (32 mg, 0.1 mmol), CH_2Cl_2 (6 mL) and NaOH (50%, 6 mL) was added benzyl chloride (278 mg, 2.2 mmol). The mixture was stirred at room temperature for 4 h, diluted with water (10 mL), and extracted with CH_2Cl_2 (3 × 5 mL), and the organic phase was dried over $MgSO_4$. The solvent was evaporated under vacuum and the residue chromatographed. Elution with EtOAc afforded 240 mg (47%) of a cream-colored solid: mp 101−102 °C (EtOH); IR (KBr) ν_{max} 3030, 1587, 1450, 1358, 1334, 1166 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.35 (s, 1H), 8.53 (d, 1H, J = 5.6 Hz), 8.18 (d, 1H, J = 7.8 Hz), 7.49−7.13 (m, 7H), 7.12−7.10 (m, 2H), 5.49 (s, 2H) ppm. Anal. Calcd for $C_{18}H_{14}N_2$: C, 83.69; H, 5.46; N, 10.84. Found: C, 83.54; H, 5.40; N, 10.98.

General Procedure for the Preparation of Pyridylbenzotriazoles. Method A. An equimolar mixture of the 4-chloroazine derivative (or its hydrochloride) and the benzotriazole derivative (2 mmol) was heated at 150−210 °C for 15−30 min. The reaction mixture was allowed to cool to room temperature, and the solid formed was purified by recrystallization or, if necessary, by column chromatography.

Method B. The same mixture of the benzotriazole derivative and 4-chloroazine employed in method A was placed in an open flask and irradiated in a commercial microwave oven at 160 W for 7−10 min. The reaction mixture was worked up as indicated above.

1-(2-Methylpyridin-4-yl)-1*H*-benzotriazole (14a): mp 92−93 °C (hexane/EtOAc, brown prisms); IR (KBr) ν_{max} 1601, 1490, 1452, 1294, 1227, 1064, 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.73 (d, 1H, J = 5.5 Hz), 8.19 (dt, 1H, J = 8.3 Hz, J = 1.0 Hz, J = 0.9 Hz), 7.87 (dt, 1H, J = 8.4 Hz, J = 1.0 Hz, J = 0.9 Hz), 7.73 (s, 1H), 7.66−7.61 (m, 2H), 7.49 (ddd, 1H, J = 8.3 Hz, J = 7.1 Hz, J = 0.9 Hz), 2.72 (s, 3H) ppm; MS (EI) m/z (rel int) 210 (M^+, 21), 182 (53), 154 (30), 92 (97), 77 (8), 65 (100). Anal. Calcd for $C_{12}H_{10}N_4$: C, 68.56; H, 4.79; N, 26.65. Found: C, 68.69; H, 4.91; N, 26.40.

5,6-Dimethyl-1-(2-methylpyridin-4-yl)-1*H*-benzotriazole (14b): 94−95 °C (hexane/EtOAc, white prisms); IR (KBr) ν_{max} 1597, 1490, 1466, 1309, 1228, 1057, 1023, 1009 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.66 (d, 1H, J = 5.4 Hz), 7.94 (s, 1H), 7.92 (s, 1H), 7.83 (s, 1H), 7.78 (d, 1H, J = 5.4 Hz), 2.61 (s, 3H), 2.43 (s, 3H), 2.38 (s, 3H) ppm; MS (EI) m/z (rel int) 238 (M^+, 38), 210 (55), 209 (100), 195 (30), 118 (8), 92 (25). Anal. Calcd for $C_{14}H_{14}N_4$: C, 70.57; H, 5.92; N, 23.51. Found: C, 70.35; H, 5.74;N, 23.40.

1-Pyridin-4-yl-1*H*-benzotriazole hydrochloride (14c): mp 231−232 °C (EtOH/H_2O, white powder) (lit.[53] mp 116−117 °C, free base, hexane/EtOAc); IR (KBr) ν_{max} 3409, 1628, 1600, 1509, 1177, 1028 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 9.01 (d, 2H, J = 5.9 Hz), 8.42 (d, 2H, J = 5.9 Hz), 8.28 (d, 2H, J = 8.7 Hz), 7.80 (ddd, 1H, J = 8.2 Hz, J = 7.2 Hz, J = 1.2 Hz), 7.62 (ddd, 1H, J = 8.3 Hz, J = 7.2 Hz, J = 1.2 Hz) ppm; MS (EI) m/z (rel int) 196 (M^+, 51), 168 (100), 140 (28), 91 (12), 78 (62). Anal. Calcd for $C_{11}H_8N_4$·HCl: C, 56.78; H, 3.90; N, 24.08. Found: C, 57.01; H, 3.63; N, 23.89.

1-(2,6-Dimethylpyridin-4-yl)-1*H*-benzotriazole (14d): mp 112−113 °C (EtOH/H_2O) (lit.[47] mp 114−115 °C, EtOH).

5,6-Dimethyl-1-pyridin-4-yl-1*H*-benzotriazole hydrochloride (14e): mp 258−259 °C (EtOH/H_2O, white powder) (lit.[47] mp 144−145 °C, free base, EtOH/H_2O); IR (KBr) ν_{max} 2448, 1632, 1608, 1311, 1031, 1005 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.99 (d, 2H, J = 6.8 Hz), 8.42 (d, 2H, J = 6.8 Hz), 8.10 (s, 1H), 7.99 (s, 1H), 5.2 (bs, 1H), 2.46 (s, 3H), 2.40 (s, 3H) ppm; MS (EI) m/z (rel int) 224 (M^+, 30), 196 (47), 195 (100), 181 (30), 118 (11), 78 (25). Anal. Calcd for $C_{13}H_{12}N_4$·HCl: C, 59.89; H, 5.03; N, 21.49. Found: C, 59.75; H, 5.21; N, 21.42.

1-(2,6-Dimethyl-1-pyridin-4-yl)-5,6-dimethyl-1*H*-benzotriazole (14f): mp 128−129 °C (hexane/EtOAc, cream-colored prisms); IR (KBr) ν_{max} 1594, 1486, 1454, 1240, 1058 cm^{-1}; ^1H NMR (DMSO-d_6) δ 7.83 (s, 2H), 7.54 (s, 2H), 2.53 (s, 6H), 2.38 (s, 3H), 2.32 (s, 3H) ppm; MS (EI) m/z (rel int) 252 (M^+, 45), 224 (61), 223 (100), 209 (25), 118 (22), 106 (51), 91 (28). Anal. Calcd for $C_{15}H_{16}N_4$: C, 71.40; H, 6.39; N, 22.20. Found: C, 71.71; H, 6.25; N, 22.42.

4-Benzotriazol-1-ylquinoline Hydrochloride (15a): mp 176−177 °C (EtOH/H_2O, white prisms) (lit.[48] mp 132−133 °C, free base, EtOH); IR (KBr) ν_{max} 3396, 1588, 1512, 1486, 1452, 1433, 1000 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.23 (d, 1H, J = 4,9 Hz), 8.29 (d, 2H, J = 8.3 Hz), 8.00−7.94 (m, 2H), 7.79−7.64 (m, 4H), 7.58 (ddd, 1H, J = 8.2 Hz, J = 7.0 Hz, J = 1.2 Hz) ppm; MS (EI) m/z (rel int) 246 (M^+, 31), 218 (100), 128 (53), 101 (40), 90 (7). Anal. Calcd for $C_{15}H_{10}N_4$·HCl: C, 63.72; H, 3.92; N, 19.82. Found: C, 63.61; H, 4.06; N, 19.61.

4-Benzotriazol-1-yl-2-methylquinoline hydrochloride (15b): mp 203−204 °C (EtOAc/EtOH, purple prisms) (lit.[49] mp 210 °C, H_2O).

4-(5,6-Dimethylbenzotriazol-1-yl)quinoline hydrochloride (15c): mp 198−189 °C (EtOH, white prisms); IR (KBr) ν_{max} 3430, 1628, 1594, 1496, 1484, 1436, 1422, 998 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.26 (d, 1H, J = 4.9 Hz), 8.33 (d, 1H, J = 8.3 Hz), 8.03−7.97 (m, 3H), 7.85 (d, 1H, J = 8.4 Hz), 7.76 (ddd, 1H, J = 8.3 Hz, J = 7.0 Hz, J = 1.2 Hz), 7.50 (s, 1H), 2.41 (s, 3H), 2.36 (s, 3H) ppm; MS (EI) m/z (rel int) 274 (M^+, 31), 246 (65), 245 (100), 231 (58), 128 (62), 101 (51). Anal. Calcd for $C_{17}H_{14}N_4$·HCl: C, 65.70; H, 4.86; N, 18.03. Found: C, 65.98; H, 4.70; N, 18.22.

4-(5,6-Dimethylbenzotriazol-1-yl)-2-methylquinoline hydrochloride (15d): mp 212−213 °C (EtOH, purple prisms); IR (KBr) ν_{max} 3402, 1653, 1596, 1501, 1379, 985 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.32 (d, 1H, J = 8.4 Hz), 8.04−7.98 (m, 3H), 7.87 (d, 1H, J = 8.1 Hz), 7.74 (t, 1H, J = 8.4 Hz, J = 7.6 Hz), 7.57 (s, 1H), 2.92 (s, 3H), 2.41 (s, 3H), 2.36 (s, 3H) ppm; MS (EI) m/z (rel int) 288 (M^+, 44), 260 (100), 245 (64), 142 (61), 115 (25), 101 (41). Anal. Calcd for $C_{18}H_{16}N_4$·HCl: C, 66.56; H, 5.28; N, 17.25. Found: C, 66.21; H, 5.20; N, 17.32.

1-(2-Methylpyridin-4-yl)-1*H*-naphtho[2,3-*d*][1,2,3]triazole (16a): mp 153−154 °C (hexane-/EtOAc, orange powder); IR (KBr) ν_{max} 1593, 1566, 1486, 1321, 1048, 1015 cm^{-1}; ^1H NMR (DMSO-d_6) δ 8.94 (s, 1H), 8.81 (s, 1H), 8.72 (d, 1H, J = 5.4 Hz), 8.24−8.20 (m, 2H), 8.02 (s, 1H), 7.96 (d, 1H, J = 5.4 Hz), 7.64 (ddd, 1H, J = 8.3 Hz, J = 6.8 Hz, J = 1.4 Hz), 7.57 (ddd, 1H, J = 8.3 Hz, J = 6.8 Hz, J = 1.4 Hz), 2.66 (s, 3H) ppm; MS (EI) m/z (rel int) 260 (M^+, 41), 232 (100), 140 (15), 92 (18). Anal. Calcd for $C_{16}H_{12}N_4$: C, 73.83; H, 4.65; N, 21.52. Found: C, 73.98; H, 4.42; N, 21.60.

1-Pyridin-4-yl-1*H*-naphtho[2,3-*d*][1,2,3]triazole hydrochloride (16b): mp 230−231 °C (EtOH/H_2O, brown powder); IR (KBr) ν_{max} 3396, 1602, 1516, 1007 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.99−8.97 (m, 3H), 8.89 (s, 1H), 8.41 (d, 2H, J = 5.4 Hz), 8.26−8.20 (m, 2H), 7.68 (ddd, 1H, J = 8.3 Hz, J = 6.7 Hz, J = 1.5 Hz), 7.60 (ddd, 1H, J = 8.3 Hz, J = 6.7 Hz, J = 1.5 Hz) ppm; MS (EI) m/z (int relat) 246 (M^+, 30), 218 (100), 140 (20), 78 (9). Anal. Calcd for $C_{15}H_{10}N_4$·HCl: C, 63.72; H, 3.92; N, 19.82. Found: C, 63.54; H, 4.11; N, 19.93.

1-Quinolin-4-yl-1*H*-naphtho[2,3-*d*][1,2,3]triazole Hydrochloride (17a): mp 222−223 °C (EtOH, white powder); IR (KBr) ν_{max} 1594, 1566, 1510, 1432, 1405, 1036 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.23 (d, 1H, J = 4.6 Hz), 9.01 (s, 1H), 8.29−8.24 (m, 3H), 8.08 (dd, 1H, J = 8.2 Hz, J = 1.5 Hz), 8.04 (d, 1H, J = 4.6 Hz), 7.94 (ddd, 1H, J = 8.5 Hz, J = 6.9 Hz, J = 1.5 Hz), 7.83 (dd, 1H, J = 7.9 Hz, J = 1.2 Hz), 7.70 (ddd, 1H, J = 8.4 Hz, J = 6.8 Hz, J = 1.4 Hz), 7.61−7.52 (m, 2H) ppm; MS (EI) m/z (rel int) 296 (M^+, 19), 268 (100), 128 (28), 101 (34). Anal. Calcd for $C_{19}H_{12}N_4$·HCl: C, 68.57; H, 3.94; N, 16.84. Found: C, 68.45; H, 4.12; N, 16.93.

1-(2-Methylquinolin-4-yl)-1*H*-naphtho[2,3-*d*][1,2,3]-triazole hydrochloride (17b): mp 214−215 °C (EtOH/H_2O, brown powder); IR (KBr) ν_{max} 3478, 1637, 1595, 1503, 1433, 1033 cm^{-1}; ^1H NMR (DMSO-d_6) δ 9.03 (s, 1H), 8.35 (s, 1H), 8.30−8.25 (m, 2H), 8.12−8.09 (m, 2H), 8.01−7.92 (m, 2H), 7.71 (t, 1H, J = 7.7 Hz, J = 7.6 Hz), 7.63−7.54 (m, 2H), 2.91 (s, 3H) ppm; MS (EI) m/z (rel int) 310 (M^+, 23), 282 (100), 142 (17), 140 (22), 101 (26). Anal. Calcd for $C_{20}H_{14}N_4$·HCl: C, 69.26; H, 4.36;N, 16.15. Found: C, 69.52; H 4.11; N, 16.40.

General Procedure for the Preparation of γ-Carbolines Derivatives 18−25. Method A. A mixture of the corresponding pyridylbenzotriazole derivative **14**−**17** (2 mmol) and $H_4P_2O_7$ (6 mmol) was heated at 150 °C until the evolution of nitrogen ceased (1.5−2 h). The reaction mixture was then triturated with water and basified with a 15% solution of NaOH. The precipitate formed was filtered off and purified by recrystallization or, if neccessary, by column chromatography.

Method B. A mixture of the corresponding pyridylbenzotriazole derivative (2 mmol) and $H_4P_2O_7$ (6 mmol) was placed in an open flask and irradiated in a commercial microwave oven at 160 W for 4−6 min. The reaction mixture was then worked up as indicated above in method A.

Method C. An equimolar mixture of the corresponding benzotriazole derivative and 4-chloroazine (2 mmol) was placed in an open flask and then irradiated in a commercial microwave oven at 160 W for 7−10 min. The reaction mixture was allowed to cool to room temperature, and $H_4P_2O_7$ (16 mmol) was added. Microwave irradiation was continued at the same power for 4−6 min. The resulting reaction mixture was purified as indicated in methods A and B.

1-Methyl-5*H*-pyrido[4,3-*b*]indole (18a). A mixture of **18a** and **19a** was chromatographed (CH_2Cl_2/acetone, 9.5:0.5) to give 102 mg of the title compound: mp 248−249 °C (CH_3CN, cream-colored powder); IR (KBr) ν_{max} 2670, 1600, 1579, 1335, 1220, 998 cm^{-1}.^1H NMR (DMSO-d_6) δ 11.70 (bs, 1H, NH), 8.25 (d, 1H, H_3, J_{3-4} = 5.7 Hz), 8.12 (d, 1H, H_9, J_{9-8} = 7.7 Hz), 7.55 (d, 1H, H_6, J_{6-7} = 8.1 Hz), 7.45 (ddd, 1H, H_7, J_{7-6} = 8.1 Hz, J_{7-8} = 7.1 Hz, J_{7-9} = 1.2 Hz), 7.32−7.24 (m, 2H, H_4 and H_8), 2.91 (s, 3H) ppm; MS (EI) m/z (rel int) 182 (M^+, 100), 181 (21), 154 (22). Anal. Calcd for $C_{12}H_{10}N_2$: C, 79.10; H, 5.53; N, 15.37. Found: C, 79.35; H, 5.62; N, 15.03.

3-Methyl-5*H*-pyrido[4,3-*b*]indole (19a). Elution with CH_2Cl_2/acetone (7:3) afforded 124 mg of the title compound: mp 226−227 °C (EtOAc, orange prisms) (lit.[50] mp 225−228 °C, benzene).

1,7,8-Trimethyl-5*H*-pyrido[4,3-*b*]indole (18b). A mixture of **18b** and **19b** was chromatographed (CH_2Cl_2/acetone, 9.5:0.5) to give 176 mg of the title compound: mp 272−273 °C (CH_3CN, cream-colored powder); IR (KBr) ν_{max} 2919, 1595, 1578, 1448, 1279, 1221, 1124 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.45 (bs, 1H, NH), 8.18 (d, 1H, H_3, J_{3-4} = 5.7 Hz), 7.86 (s, 1H, H_9), 7.32 (s, 1H, H_6), 7.23 (d, 1H, H_4, J_{4-3} = 5.7 Hz), 2.88 (s, 3H), 2.38 (s, 3H), 2.37 (s, 3H) ppm; MS (EI) m/z (rel int) 210 (M^+, 100), 209 (46), 195 (44). Anal. Calcd for $C_{14}H_{14}N_2$: C, 79.97; H, 6.71; N, 13.22. Found: C, 80.13; H, 6.90; N, 12.97.

3,7,8-Trimethyl-5*H*-pyrido[4,3-*b*]indole (19b). Elution with CH_2Cl_2/acetone (7:3) afforded 199 mg of the title compound: mp 308−309 °C (EtOAc, pale yellow prisms); IR (KBr) ν_{max} 2718, 1617, 1579, 1465, 1452, 1255, 1238, 1159 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.28 (bs, 1H, NH), 9.04 (s, 1H, H_1), 7.88 (s, 1H, H_9), 7.26 (s, 1H, H_6), 7.19 (s, 1H, H_4), 2.53 (s, 3H), 2.34 (s, 3H), 2.33 (s, 3H) ppm; MS (EI) m/z (relat int) 210 (M^+,

(47) Nantka-Namirski, P.; Zieleniak, J. *Acta Polon. Pharm.* **1977**, *34*, 349.
(48) Kermack, W. O.; Storey, N. E. *J. Chem. Soc.* **1950**, 607.
(49) Kermack, W. O.; Smith, J. F. *J. Chem Soc.* **1930**, 1999.
(50) Ducrocq, C.; Civier, A.; André-Luisfert, J.; Bisagni, E. *J. Heterocycl. Chem.* **1975**, *12*, 963.

100), 209 (47), 195 (46). Anal. Calcd for $C_{14}H_{14}N_2$: C, 79.97; H, 6.71; N, 13.22. Found: C, 79.68; H, 6.66;N, 13.60.

5*H*-Pirido[4,3-*b*]indole (20a): mp 230−231 °C (CH_3CN, cream-colored needles) (lit.[51] mp 230−231 °C, MeOH/H_2O).

7,8-Dimethyl-5*H*-pyrido[4,3-*b*]indole (20b): mp 250−251 °C (CH_3CN, cream-colored needles) (lit.[51] mp 251−252 °C (EtOH/H_2O)).

1,3-Dimethyl-5*H*-pyrido[4,3-*b*]indole (20c): mp 241−242 °C (EtOAc/EtOH, cream-colored powder) (lit.[52] mp 241−242 °C (EtOH/H_2O)).

1,3,7,8-Tetramethyl-5*H*-pyrido[4,3-*b*]indole (20d): mp 296−297 °C (CH_3CN, cream-colored powder); IR (KBr) ν_{max} 2921, 1607, 1579, 1465, 1292, 1276, 1227, 1166 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.24 (bs, 1H), 7.79 (s, 1H, H_9), 7.26 (s, 1H, H_6), 7.06 (s, 1H, H_4), 2.83 (s, 3H), 2.50 (s, 3H), 2.37 (s, 3H), 2.36 (s, 3H) ppm; MS (EI): *m*/*z* (rel int) 224 (M^+, 100), 223 (36), 209 (37). Anal. Calcd for $C_{15}H_{16}N_2$: C, 80,32; H, 7.19; N, 12.49. Found: C, 80.57; H, 7.29; N, 12.76.

11*H*-Indolo[3,2-*c*]quinoline (21a): mp 336−337 °C (CH_3CN, cream-colored powder) (lit.[52] mp 333 °C (MeOH)).

6-Methyl-11*H*-indolo[3,2-*c*]quinoline (21b): mp 295−296 °C (CH_3CN, cream-colored powder) (lit.[49] mp 298 °C, MeOH); IR (KBr) ν_{max} 3042, 1563, 1514, 1453, 1360, 1319, 1247, 1208 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.70 (bs, 1H, NH), 8.46 (d, 1H, H_4, J_{4-3} = 8.0 Hz), 8.21 (d, 1H, H_7, J_{7-8} = 7.7 Hz), 8.02 (d, 1H, H_1, J_{1-2} = 8.1 Hz), 7.72−7.65 (m, 2H, H_2 and H_{10}), 7.60 (t, 1H, H_3, J_{3-4} = 8.0 Hz, J_{3-2} = 7.1 Hz), 7.48 (t, 1H, H_9, J_{9-8} = 7.3 Hz, J_{9-10} = 7.8 Hz), 7.32 (t, 1H, H_8, J_{8-7} = 7.7 Hz, J_{8-9} = 7.3 Hz), 3.06 (s, 3H) ppm; ^{13}C NMR (DMSO-d_6) δ 154.21, 144.86, 139.82, 138.69, 128.62, 127.94, 124.93, 124.78, 122.26, 121.72, 121.50, 120.56, 116.18, 112.88, 111.72, 24.45 ppm; MS (EI) *m*/*z* (rel int) 232 (M^+, 100), 231 (24), 204 (11). Anal. Calcd for $C_{16}H_{12}N_2$: C, 82.73; H 5.21; N 12.06. Found: C, 82.53; H, 5.35; N, 12.12.

8,9-Dimethyl-11*H*-indolo[3,2-*c*]quinoline (21c): mp 360−361 °C (EtOH, cream-colored prisms); IR (KBr) ν_{max} 2922, 1630, 1570, 1514, 1504, 1466, 1362, 1279, 1258, 1245 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.45 (bs, 1H), 9.48 (s, 1H, H_6), 8.46 (d, 1H, H_4, J_{4-3} = 7.7 Hz), 8.08 (d, 1H, H_1, J_{1-2} = 8.2 Hz), 8.04 (s, 1H, H_7), 7.69−7.63 (m, 2H, H_2 and H_3), 7.47 (s, 1H, H_{10}), 2.42 (s, 3H), 2.40 (s, 3H) ppm; MS (EI) *m*/*z* (rel int) 246 (M^+, 100), 245 (44). Anal. Calcd for $C_{17}H_{14}N_2$: C, 82.90; H, 5.73; N, 11.37. Found: C, 83.24;H, 5.61; N, 11.15.

6,8,9-Trimethyl-11*H*-indolo[3,2-*c*]quinoline (21d): mp 330−331 °C (EtOH, cream-colored prisms); IR (KBr) ν_{max} 2954, 2799, 1566, 1516, 1390, 1351, 1269, 1249, 1209, 1116 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.44 (bs, 1H, 8.43 (dd, 1H, H_4, J_{4-3} = 8.0 Hz, J_{4-2} = 1.6 Hz), 7.99 (d, 1H, H_1, J_{1-2} = 8.2 Hz), 7.95 (s, 1H, H_7), 7.64 (ddd, 1H, H_2, J_{2-1} = 8.2 Hz, J_{2-3} = 6,9 Hz, J_{2-4} = 1.6 Hz), 7.57 (ddd, 1H, H_3, J_{3-4} = 8.0 Hz, J_{3-2} = 6.9 Hz, J_{3-1} = 1.5 Hz), 7.47 (s, 1H, H_{10}), 3.04 (s, 3H), 2.42 (s, 6H) ppm; MS (EI) *m*/*z* (rel int) 260 (M^+, 100), 259 (33), 245 (29). Anal. Calcd for $C_{18}H_{16}N_2$: C, 83.04; H, 6.19; N, 10.76. Found: C, 82.74; H, 6.46; N, 10.45.

1-Methyl-5*H*-benzo[*f*]pyrido[4,3-*b*]indole (22). A mixture of **22** and **23** was chromatographed (CH_2Cl_2/acetone, 9.5:0.5) to give 177 mg of the title compound: mp 294−295 °C (CH_3CN, brown prisms); IR (KBr) ν_{max} 3429, 2927, 1644, 1593, 1482, 1415, 1263, 1227, 1184 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.66 (bs, 1H, NH), 8.69 (s, 1H, H_{11}), 8.34 (d, 1H, H_3, J_{3-4} = 5.6 Hz), 8.16 (d, 1H, H_{10}, J_{10-9} = 8.4 Hz), 8.02 (d, 1H, H_7, J_{7-8} = 8.1 Hz), 7.93 (s, 1H, H_6), 7.49 (t, 1H, H_8, J_{8-7} = 8.1 Hz, J_{8-9} = 6.9 Hz), 7.41 (t, 1H, H_9, J_{9-10} = 8.4 Hz, J_{9-8} = 6.9 Hz), 7.31 (d, 1H, H_4, J_{4-3} = 5.6 Hz), 3.03 (s, 3H) ppm; MS (EI) *m*/*z* (rel int) 232 (M^+, 100), 204 (16). Anal. Calcd for $C_{16}H_{12}N_2$: C, 82.73; H, 5.21; N, 12.06. Found: C, 82.60; H, 5.34; N, 12.06.

3-Methyl-5*H*-benzo[*f*]pyrido[4,3-*b*]indole (23). Elution with CH_2Cl_2/acetone (7:3) afforded 192 mg of the title compound: mp 308−309 °C (CH_3CN, cream-colored powder); IR (KBr) ν_{max} 3401, 3043, 1618, 1477, 1446, 1236, 1158 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.51 (bs, 1H, NH), 9.26 (s, 1H, H_1), 8.71 (s, 1H, H_{11}), 8.05−7.97 (m, 2H, H_7 and H_{10}), 7.87 (s, 1H, H_6), 7.47 (t, 1H, H_8, J_{8-7} = 8.1 Hz, J_{8-9} = 7.0 Hz), 7.38 (t, 1H, H_9, J_{9-8} = 7.0 Hz, J_{9-10} = 8.3 Hz), 7.27 (s, 1H, H_4), 2.59 (s, 3H) ppm; MS (EI) *m*/*z* (rel int) 232 (M^+, 100), 204 (15). Anal. Calcd for $C_{16}H_{12}N_2$: C, 82.73; H, 5.21; N, 12.06. Found: C, 82.45;H, 5.14; N, 12.37.

5*H*-Benzo[*f*]pyrido[4,3-*b*]indole (24): mp 281−282 °C (MeOH/H_2O, brown powder); IR (KBr) ν_{max} 2715, 1608, 1576, 1484, 1267, 1241 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.65 (bs, 1H), 9.41 (s, 1H, H_1), 8.79 (s, 1H, H_{11}), 8.47 (d, 1H, H_3, J_{3-4} = 5,6 Hz), 8.07 (d, 1H, H_{10}, J_{10-9} = 8.3 Hz), 8.03 (d, 1H, H_7, J_{7-8} = 8.1 Hz), 7.93 (s, 1H, H_6), 7.49 (ddd, 1H, H_8, J_{8-7} = 8.1 Hz, J_{8-9} = 6,8 Hz, J_{8-10} = 1.8 Hz), 7.44−7.39 (m, 2H, H_4 and H_9) ppm; ^{13}C NMR (DMSO-d_6) δ 146.54, 146.32, 142.97, 139.01, 132.45, 128.23, 128.08, 127.06, 125.27, 123.01, 122.94, 119.12, 119.00, 105.96 ppm; MS (EI) *m*/*z* (rel int) 218 (M^+, 100), 190 (14), 163 (13), 109 (42). Anal. Calcd for $C_{15}H_{10}N_2$: C, 82.55; H, 4.62; N, 12.83: Found: C, 82.35; H, 4.60; N, 13.05.

13*H*-Benz[5,6]indolo[3,2-*c*]quinoline (25a): mp 342−343 °C (MeOH/H_2O, brown powder); IR (KBr) υ_{max} 3028, 1588, 1567, 1514, 1487, 1449, 1362, 1261, 1240, 1220 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.68 (bs, 1H), 9.68 (s, 1H, H_6), 8.88 (s, 1H, H_7), 8.56 (d, 1H, H_4, J_{4-3} = 7.7 Hz), 8.16−8.10 (m, 4H, H_1, H_8, H_{11}, and H_{12}), 7.81−7.68 (m, 2H, H_2 and H_3), 7.53−7.42 (m, 2H, H_9 and H_{10}) ppm; ^{13}C NMR (DMSO-d_6) δ 146.48, 144.88, 143.49, 138.73, 131,88, 129.70, 128.90, 128.66, 128.29, 127.47, 125.79, 125.04, 123.92, 123.36, 122.63, 118.26, 116.76, 113.87, 106.87 ppm; MS (EI) *m*/*z* (rel int) 268 (M^+, 100), 240 (10). Anal. Calcd for $C_{19}H_{12}N_2$: C, 85.05; H, 4.51; N, 10.44. Found: C, 84.97; H, 4.65; N, 10.38.

6-Methyl-13*H*-benz[5,6]indolo[3,2-*c*]quinoline (25b): mp 330−331 °C (CH_3CN, cream-colored powder); IR (KBr) ν_{max} 3432, 3051, 1627, 1569, 1518, 1430, 1367, 1250 cm^{-1}; ^1H NMR (DMSO-d_6) δ 12.65 (bs, 1H), 8.78 (s, 1H, H_7), 8.51 (d, 1H, H_4, J_{4-3} = 8.0 Hz), 8.19 (d, 1H, H_8, J_{8-9} = 7.9 Hz), 8.13−8.09 (m, 2H, H_{11} and H_{12}), 8.06 (d, 1H, J_{1-2} = 8.4 Hz), 7.74 (t, 1H, H_2, J_{2-1} = 8.4 Hz, J_{2-3} = 7.1 Hz), 7.63 (t, 1H, H_3, J_{3-2} = 7.1 Hz, J_{3-4} = 8.0 Hz), 7.52−7.42 (m, 2H, H_9 and H_{10}), 3.17 (s, 3H) ppm; MS (EI) *m*/*z* (rel int) 282 (M^+, 100). Anal. Calcd for $C_{20}H_{14}N_2$: C, 85.08; H, 5.00; N, 9.92. Found: C, 84.90; H, 5.12; N, 9.98.

General Procedure for the N-Methylation of γ-Carboline Derivatives. To a suspension of the γ-carboline derivative (1 mmol) in acetonitrile (20 mL) was added methyl iodide (0.62 mL, 10 mmol), and the reaction mixture was stirred at room temperature (reflux was necessary for **27d**) for the time indicated below. The precipitate obtained was isolated by filtration and purified by recrystallization.

2-Methyl-5*H*-pyrido[4,3-*b*]indol-2-inium Iodide (26). After the solution was stirred for 24 h, the solid was recrystallized from EtOH/EtOAc to give 294 mg (95%) of a cream-colored powder: mp 240−241 °C (lit.[48] mp 232 °C, *i*-PrOH/EtOAc); IR (KBr) ν_{max} 3368, 1651, 1612, 1499, 1454, 1348, 1206 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.01 (bs, 1H, NH), 9.82 (s, 1H), 8.65 (d, 1H, *J* = 7.0 Hz), 8.34 (d, 1H, *J* = 7.9 Hz), 8.01 (d, 1H, *J* = 7.0 Hz), 7.78 (d, 1H, *J* = 8.2 Hz), 7.69 (t, 1H, *J* = 8.2 Hz, *J* = 7.3 Hz), 7.49 (t, 1H, J = 7.3 Hz, *J* = 7.9 Hz), 4.36 (s, 3H) ppm; MS (EI) *m*/*z* (rel int) 183 (M^+, 2), 182 (18), 168 (100), 142 (37), 128 (35). Anal. Calcd for $C_{12}H_{11}IN_2$: C, 46.47; H, 3.57; N, 9.03. Found: C, 46.39; H, 3.32; N, 9.12.

5-Methyl-11*H*-indolo[3,2-*c*]quinolin-5-inium Iodide (27a). After the solution was stirred for 24 h, the cream-colored solid was crystallized from MeOH affording 331 mg (92%): mp 298−299 °C (lit.[48] mp 297 °C, MeOH); IR (KBr) ν_{max} 3420, 3053, 1630, 1608, 1544, 1512, 1456, 1350, 1230, 1194, 1118 cm^{-1}; ^1H NMR (DMSO-d_6) δ 13.95 (bs, 1H), 10.21 (s, 1H), 8.83 (d, 1H, *J* = 8.2 Hz), 8.48 (d, 1H, *J* = 8.4 Hz), 8.36 (d, 1H, *J* = 8.1 Hz), 8.17 (t, 1H, *J* = 8.4 Hz, J = 7.3 Hz), 8.07 (t, 1H, *J* = 8.2 Hz, *J* = 7.3 Hz), 7.90 (d, 1H, *J* = 8.4 Hz), 7.71 (t, 1H, *J* = 8.4 Hz, *J* = 7.0 Hz), 7.57 (t, 1H, *J* = 8.1 Hz, J = 7.0 Hz), 4.56 (s, 3H) ppm; MS (EI) *m*/*z* (rel int) 232 (M^+, 51), 218 (33), 127 (100). Anal. Calcd for $C_{18}H_{17}IN_2$: C, 53.35; H, 3.64; N, 7.78. Found: C, 53.62; H, 3.52; N, 7.90.

5,8,9-Trimethyl-11*H*-indolo[3,2-*c*]quinolin-5-inium Iodide (27c). After the solution was stirred for 48 h, the solid obtained was recrystallized from MeOH affording 345 mg

(51) Nantka-Namirski, P.; Zieleniak, J. *Acta Polon. Pharm.* **1977**, *34*, 449.

(52) Roussel, O.; Buu-Hoï, N. P.; Jacquignon, P. *J. Chem. Soc.* **1965**, 5458.

(89%) of a cream-colored powder: mp 343−344 °C; IR (KBr) ν_{max} 3438, 3031, 1627, 1612, 1546, 1465, 1435, 1387, 1372, 1331, 1121 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 13.85 (bs, 1H, NH), 10.06 (s, 1H), 8.78 (d, 1H, J = 8.2 Hz), 8.42 (d, 1H, J = 8.6 Hz), 8.12 (t, 1H, J = 8.6 Hz, J = 7.3 Hz), 8.08 (s, 1H), 8.02 (t, 1H, J = 8.2 Hz, J = 7.3 Hz), 7.63 (s, 1H), 4.52 (s, 3H), 2.47 (s, 6H) ppm; MS (EI) m/z (rel int) 261 (M$^+$, 20), 260 (100), 245 (40), 128 (68). Anal. Calcd for $C_{18}H_{17}IN_2$: C, 55.69; H, 4.41; N, 7.22. Found: C, 56.00; H, 4.44; N, 7.22.

5,6,8,9-Tetramethyl-11*H*-indolo[3,2-*c*]quinolin-5-inium Iodide (27d). After a 10 h reflux, the solid obtained was crystallized from MeOH affording 326 mg (81%) of yellow prisms: mp 360−361 °C; IR (KBr) ν_{max} 3438, 3036, 1625, 1595, 1541, 1467, 1441, 1385, 1353, 1336, 1301, 1260, 1214 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 13.73 (bs, 1H, NH), 8.75 (d, 1H, J = 8.4 Hz), 8.51 (d, 1H, J = 8.8 Hz), 8.21 (s, 1H), 8.09 (t, 1H, J = 8.8 Hz, J = 7.3 Hz), 7.98 (t, 1H, J = 8.4 Hz, J = 7.3 Hz), 7.62 (s, 1H), 4.40 (s, 3H), 3.42 (s, 3H), 2.47 (s, 6H) ppm; MS (EI) m/z (rel int) 275 (M$^+$, 20), 274 (100), 259 (38), 128 (58). Anal. Calcd for $C_{19}H_{19}IN_2$: C, 56.73; H, 4.76; N 6.96. Found: C, 57.00; H, 4.48; N, 7.13.

2-Methyl-5*H*-benzo[f]pyrido[4,3-*b*]indol-2-inium Iodide (28). After the solution was stirred for 24 h, the solid obtained was crystallized from EtOH/EtOAc affording 345 mg (75%) of a brown powder: mp 228−229 °C; IR (KBr) ν_{max} 3440, 1632, 1594, 1494, 1245, 1231, 1208 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 13.03 (bs, 1H, NH), 9.80 (s, 1H), 8.93 (s, 1H), 8.69 (d, 1H, J = 7.0 Hz), 8.20−8.15 (m, 3H), 7.94 (d, 1H, J = 7.0 Hz), 7.63 (t, 1H, J = 8.2 Hz, J = 6.8 Hz), 7.56 (t, 1H, J = 8.1 Hz, J = 6.8 Hz), 4.36 (s, 3H) ppm; MS (EI) m/z (rel int) 233 (M$^+$, 2), 232 (11), 218 (100), 190 (14). Anal. Calcd for $C_{16}H_{13}IN_2$: C, 53.35; H, 3.64; N, 7.78. Found: C, 53.12; H, 3.42; N, 7.92.

5-Methyl-13*H*-benz[5,6]indolo[3,2-*c*]quinolin-5-inium Iodide (29). After the solution was stirred for 48 h, the solid obtained was recrystallized from MeOH affording 322 mg (78%) of yellow prisms: mp 334−335 °C (MeOH); IR (KBr) ν_{max} 3422, 3046, 1624, 1500, 1450, 1351, 1310, 1240, 1109 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 14.07 (bs, 1H, NH), 10.23 (s, 1H), 8.92 (s, 1H), 8.86 (d, 1H, J = 8.1 Hz), 8.47 (d, 1H, J = 8.7 Hz), 8.34 (s, 1H), 8.24−8.19 (m, 3H), 8.09 (t, 1H, J = 8.01 Hz, J = 7.3 Hz), 7.67−7.56 (m, 2H), 4.55 (s, 3H) ppm. Anal. Calcd for $C_{20}H_{15}IN_2$: C, 58.55; H, 3.69; N, 6.83. Found: C, 58.70; H, 3.75; N, 7.01.

General Procedure for the Preparation of Dichloro Amides 35. To an ice-cooled solution of the corresponding diamine (10 mmol) in CH_2Cl_2 (20 mL) was slowly added chloroacetyl chloride (1.56 mL, 20 mmol) under argon followed by a slow addition of ethyldiisopropylamine (Hunig's base) (3.48 mL, 20 mmol). When the addition was completed, stirring was continued at room temperature for 2 h and then the solution was concentrated under reduced pressure and the residue was triturated with distilled water. The precipitate obtained was filtered off, washed with Et_2O, and recrystallized from acetonitrile to give diamides **35** as white prisms.

2-Chloro-*N*-[4-[(chloroacetyl)amino]butyl]acetamide (35a): 65%; mp 130−131 °C (CH_3CN); IR (KBr) ν_{max} 3323, 2940, 1639, 1550, 1454, 1434, 1397, 1320, 1263 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.18 (bs, 2H), 4.02 (s, 4H), 3.09−3.06 (m, 4H), 1.40−1.38 (m, 4H) ppm; MS (EI) m/z (rel int) 147 (92), 111 (82), 98 (100), 73 (24). Anal. Calcd for $C_8H_{14}Cl_2N_2O_2$: C, 39.85; H, 5.85; N, 11.62. Found: C, 40.01; H, 5.96; N, 11.60.

2-Chloro-*N*-[6-[(chloroacetyl)amino]hexyl]acetamide (35b): 64%; mp 135−136 °C (CH_3CN); IR (KBr) ν_{max} 3272, 2937, 1663, 1561, 1462, 1402, 1338, 1240, 1225 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.16 (bs, 2H), 4.00 (s, 4H), 3.07−3.03 (m, 4H), 1.40−1.36 (m, 4H), 1.24 (bs, 4H) ppm; MS (EI) m/z (rel int) 269 (M$^+$, 3), 174 (42), 162 (50), 106 (100), 72 (46). Anal. Calcd for $C_{10}H_{18}Cl_2N_2O_2$: C, 44.62; H, 6.74; N, 10.41. Found: C, 44.80; H, 6.66; N, 10.54.

2-Chloro-*N*-[8-[(chloroacetyl)amino]octyl]acetamide (35c): 73%; mp 123−124 °C (CH_3CN); IR (KBr) ν_{max} 3320, 2928, 1674, 1642, 1545, 1527, 1416, 1375, 1264, 1228 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.16 (bs, 2H), 4.00 (s, 4H), 3.07−3.03 (m, 4H), 1,40−1,37 (m, 4H), 1,23 (bs, 8H) ppm; MS (EI) m/z (rel int) 297 (M$^+$, 36), 106 (81), 93 (47), 72 (51). Anal. Calcd for $C_{12}H_{22}Cl_2N_2O_2$: C, 48.49; H, 7.46; N, 9.42. Found: C, 48.71; H, 7.78; N, 9.66.

General Procedure for the Preparation of Diiodo Amides 36. To a solution of NaI (1.57 g, 10.5 mmol) in dry acetone (30 mL) was added the dichloro derivative **35** (5 mmol), and the mixture was stirred at room temperature for 30 min and then at reflux temperature for 12 h. The solvent was evaporated under vacuum and the residue triturated with a saturated solution of sodium thiosulfate. The precipitate obtained was isolated by filtration, washed with Et_2O, and recrystallized from a suitable solvent to give the title compounds.

2-Iodo-*N*-[4-[(iodoacetyl)amino]butyl]acetamide (36a): 85%; mp 176−177 °C (white prisms from CH_3CN); IR (KBr) ν_{max} 3270, 3089, 1637, 1561, 1458, 1423, 1304, 1197, 1157 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.22 (bs, 2H, NH), 3.59 (s, 4H), 3.03−3.01 (m, 4H), 1,39−1,36 (m, 4H) ppm; MS (EI) m/z (rel int) 424 (M$^+$, 1), 297 (2), 238 (100), 169 (40), 127 (73), 112 (49), 71 (82). Anal. Calcd for $C_8H_{14}I_2N_2O_2$: C, 22.66; H, 3.33; N, 6.61. Found: C, 22.93; H, 3.19; N, 6.70.

2-Iodo-*N*-[6-[(iodoacetyl)amino]hexyl]acetamide (36b): 81%; mp 178−179 °C (white prisms from CH_3CN); IR (KBr) ν_{max} 3286, 3083, 2932, 2856, 1634, 1561, 1458, 1424, 1309, 1167 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.18 (bs, 2H, NH), 3.58 (s, 4H), 3.03−2.97 (m, 4H), 1.38−1.34 (m, 4H), 1.24 (bs, 4H) ppm; MS (EI) m/z (rel int) 452 (M$^+$, 1), 326 (1), 266 (100), 254 (45), 226 (3), 198 (20), 98 (51), 72 (24). Anal. Calcd for $C_{10}H_{18}I_2N_2O_2$: C, 26.57; H, 4.01; N, 6.20. Found: C, 26.65; H, 4.25; N, 6.32.

2-Iodo-*N*-[8-[(iodoacetyl)amino]octyl]acetamide (36c): 93%; mp 164−165 °C (white prisms from CH_3CN); IR (KBr) ν_{max} 3268, 3088, 2919, 2848, 1637, 1564, 1458, 1422, 1316, 1158 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 8.19 (bs, 2H, NH), 3.58 (s, 4H), 3.03−2.98 (m, 4H), 1.38−1.35 (m, 4H), 1.24 (bs, 8H) ppm; MS (EI) m/z (rel int) 481 (M$^+$, 1), 353 (29), 198 (26), 185 (28), 167 (57), 127 (100), 72 (71). Anal. Calcd for $C_{12}H_{22}I_2N_2O_2$: C, 30.02; H, 4.62; N, 5.83. Found: C; 30.25; H, 4.36; N, 5.90.

General Method for the Synthesis of Bis-Salts 30 and 32. A mixture of the corresponding benzo γ-carboline **21c** or **24** (1 mmol) and 1,1′-bis(2-chloroethyl)-4,4′-bipiperidine (**34**) (205 mg, 0.7 mmol) in DMF (6 mL) was heated at 120 °C for 24 h. The mixture was then cooled to 0 °C, and the precipitate was filtered, washed with acetone (20 mL), and dried under vacuum. The solid obtained was suspended in MeOH and treated with 2 N HCl. Elimination of the solvent under reduced pressure gave the corresponding hydrochlorides which were washed with Et_2O (3 × 6 mL), dried under vacuum, and recrystallized to give pure bis-salts **30** and **32**.

5,5′-[2,2′-(4,4′-Bipiperidin-1,1′-diyl)diethylene]bis(8,9-dimethyl-11*H*-indolo[3,2-*c*]quinolin-5-inim) tetrahydrochloride (30): 62%; mp 330−331 °C (DMF, brown powder); IR (KBr) ν_{max} 3419, 1616, 1515, 1470, 1394, 1231 cm^{-1}; ^{1}H NMR (CD_3OD): δ 14.05 (bs, 2H, NH), 10.10 (s, 2H), 8.79 (d, 2H, J = 8.1 Hz), 8.64 (d, 2H, J = 8.7 Hz), 8.250 (t, 2H, J = 8.7 Hz, J = 7.3 Hz), 8.21 (s, 2H), 8.09 (t, 2H, J = 8.1 Hz, J = 7.3 Hz), 7.70 (s, 2H), 5,59−5,57 (s, 4H), 3.90−3.78 (m, 8H), 3.24−3.20 (m, 4H), 2.58 (s, 6H), 2.57 (s, 6H), 2.11−2.06 (m, 4H), 1.72−1.64 (m, 6H), 1.50−1.48 (m, 4H), 1.27 (bs, 4H) ppm. Anal. Calcd for $C_{48}H_{54}Cl_2N_6 \cdot 3H_2O \cdot 2HCl$: C, 63.16; H, 6.85; N, 9.21. Found: C, 62.95; H, 7.15; N, 9.40.

2,2-[2,2′-(4,4′-Bipiperidin-1,1′-diyl)diethylene]bis(5*H*-benzo[*f*]pyrido[4,3-*b*]indol-2-inium) tetrahydrochloride (32): 61%; mp 310−311 °C (DMF, brown powder); IR (KBr) ν_{max} 3424, 1636, 1496, 1464, 1456, 1235, 1202 cm^{-1}; ^{1}H NMR (CD_3OD) δ 13.08 (bs, 2H, NH), 9.83 (s, 2H), 8.94 (s, 2H), 8.75 (d, 2H, J = 6.8 Hz), 8.18 (d, 2H, J = 8.2 Hz), 8.15 (s, 2H), 8.11 (d, 2H, J = 8.1 Hz), 7.93 (d, 2H, J = 6.8 Hz), 7.65 (t, 2H, J = 8.1 Hz, J = 7.0 Hz), 7.58 (t, 2H, J = 8.2 Hz, J = 7.0 Hz), 5.15−5.13 (m, 4H), 3.89−3.76 (m, 8H), 3.24−3.20 (m, 4H), 2.10−2.06 (m, 4H), 1.76−1.58 (m, 6H) ppm. Anal. Calcd for $C_{44}H_{46}Cl_2N_6 \cdot 2H_2O \cdot 2HCl$: C, 63.01; H, 6.25; N, 10.02. Found: C, 63.35; H, 6.52; N, 9.85.

General Method for the Synthesis of Bis-Salts 31 and 33. A mixture of the corresponding benzo γ-carboline **21c** or **24** (1 mmol) and the diiodo amide **36** (0.5 mmol) in DMF (3 mL) was heated at 100 °C for 6 h (for **24**) or 4 h (for **21c**). The reaction mixture was then cooled to room temperature, and

the precipitate formed (for **33** the precipitate is extensively formed by only adding of water (10 mL)) was isolated by filtration and recrystallized.

5,5′-(3,8-Diaza-2,9-dioxodecamethylene)bis(8,9-dimethyl-11*H*-indolo[3,2-*c*]quinolin-5-inium) diiodide (31a): 97%; mp 252−253 °C (DMF, brown powder); IR (KBr) ν_{max} 3214, 3059, 1686, 1648, 1625, 1549, 1470, 1392, 1234, 1102 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 16.02 (bs, 2H, NH), 10.03 (s, 2H), 8.78 (d, 2H, J = 8.1 Hz), 8.68 (bs, 2H, NH), 8.14 (d, 2H, J = 8.4 Hz), 8.07−8.03 (m, 4H), 7.97 (t, 2H, J = 8.1 Hz, J = 7.3 Hz), 7.67 (s, 2H), 5.64 (s, 4H), 3.18−3.15 (m, 4H), 2.43 (s, 6H), 2.38 (s, 6H), 1.51−1.49 (m, 4H) ppm. Anal. Calcd for $C_{42}H_{42}I_2N_6O_2$: C, 55.01; H, 4.62; N, 9.17. Found: C, 55.35; H, 4.74; N, 9.02.

5,5′-(3,10-Diaza-2,11-dioxododecamethylene)bis(8,9-dimethyl-11*H*-indolo[3,2-*c*]quinolin-5-inium) diiodide (31b): 83%; mp 254−255 °C (DMF, brown powder); IR (KBr) ν_{max} 3438, 1644, 1614, 1549, 1468, 1390, 1233, 1204 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 14.01 (bs, 2H, NH), 9.98 (s, 2H), 8.78 (d, 2H, J = 8.2 Hz), 8.65 (bs, 2H, NH), 8.14 (d, 2H, J = 8.5 Hz), 8.10 (t, 2H, J = 8.5 Hz, J = 7.3 Hz), 8.06 (s, 2H), 7.97 (t, 2H, J = 8.2 Hz, J = 7.3 Hz), 7.67 (s, 2H), 5.65 (s, 4H), 3.17−3.14 (m, 4H), 2.45 (s, 6H), 2.41 (s, 6H), 1.50−1.48 (m, 4H), 1.27 (bs, 4H) ppm. Anal. Calcd for $C_{44}H_{46}I_2N_6O_2\cdot 2H_2O$: C, 53.89; H, 5.14; N 8.57. Found: C, 53.86; H, 5.03; N, 8.58.

5,5′-(3,12-Diaza-2,13-dioxotetradecamethylene)bis(8,9-dimethyl-11*H*-indolo[3,2-*c*]quinolin-5-inium) diiodide (31c): 91%; mp 228−229 °C (DMF, brown powder); IR (KBr) ν_{max} 3219, 3053, 1682, 1657, 1616, 1547, 1468, 1389, 1233, 1102 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 14.05 (bs, 2H, NH), 10.02 (s, 2H), 8.77 (d, 2H, J = 8.1 Hz), 8.67 (bs, 2H, NH), 8.15 (d, 2H, J = 8.4 Hz), 8.10 (t, 2H, J = 8.4 Hz, J = 7.2 Hz), 8.04−7.97 (m, 4H), 7.65 (s, 2H), 5.63 (s, 4H), 3.15−3.12 (m, 4H), 2.46 (s, 6H), 2.41 (s, 6H), 1.47−1.42 (m, 4H), 1.25 (bs, 8H) ppm. Anal. Calcd for $C_{46}H_{50}I_2N_6O_2$: C, 56.80; H, 5.18; N, 8.64. Found: C, 56.39; H, 5.15; N; 9.02.

2,2′-(3,10-Diaza-2,11-dioxododecamethylene)bis(5*H*-benzo[*f*]pyrido[4,3-*b*]indol-2-inium) diiodide (33a): 96% mp 220−221 °C (DMF/H_2O, yellow powder); IR (KBr) ν_{max} 3423, 1664, 1635, 1563, 1494, 1234, 1209 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 13.05 (bs, 2H, NH), 9.43 (s, 2H), 8.78 (s, 2H), 8.58 (bs, 2H, NH), 8.30 (d, 2H, J = 6.8 Hz), 8.12 (s, 2H), 8.08 (d, 4H, J = 8.4 Hz), 7.63 (d, 2H, J = 6.8 Hz), 7.54 (t, 2H, J = 8.1 Hz, J = 7.0 Hz), 7.45 (t, 2H, J = 8.3 Hz, J = 7.0 Hz), 5.23 (s, 4H), 3.18−3.14 (m, 4H), 1.51−1.47 (m, 4H), 1.34 (bs, 4H) ppm. Anal. Calcd for $C_{40}H_{38}I_2N_6O_2\cdot H_2O$: C, 52.99; H, 4.45; N, 9.27. Found: C, 53.25; H, 4.80; N, 9.02.

2,2′-(3,12-Diaza-2,13-dioxotetradecamethylene)bis(5*H*-benzo[*f*]pyrido[4,3-*b*]indol-2-inium) diiodide (33b): 91%; mp 240−241 °C (DMF/H_2O, yellow powder); IR (KBr) ν_{max} 3419, 1666, 1634, 1556, 1494, 1372, 1234, 1209 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 13.08 (bs, 2H, NH), 9.60 (s, 2H), 8.78 (s, 2H), 8.71 (bs, 2H, NH), 8.42 (d, 2H, J = 7.0 Hz), 8.10 (s, 2H), 8.05 (d, 4H, J = 8.4 Hz), 7.74 (d, 2H, J = 7.0 Hz), 7.53 (t, 2H, J = 8.1 Hz, J = 6.9 Hz), 7.44 (t, 2H, J = 8.3 Hz, J = 6.9 Hz), 5.31 (s, 4H), 3.15−3.11 (m, 4H), 1.46−1.42 (m, 4H), 1.25−1.21 (m, 8H) ppm. Anal. Calcd for $C_{42}H_{42}I_2N_6O_2.2H_2O$: C, 52.95; H 4.87; N, 8.82. Found: C, 52.63; H, 4.75; N, 8.98.

UV−Vis Spectrophotometric Studies. All compounds examined obeyed Beer's law over the range of concentrations used (0−9 μM), and the molar extinction coefficients were determined at their appropriate ν_{max} values by Beer's law plots. Molar extinction coefficients of compounds bound to DNA were determined at the same wavelength as the molar extinction coefficient of the free compound, but a larger molar excess of DNA was present ([DNAnucleotides]/[compound] > 50−100). Spectrophotometric titrations were performed by serial addition of 50−100 μL aliquots of a DNA stock solution (0.2−0.5 mM DNAnucleotides in Tris buffer) into a 10 cm pathlength quartz cell containing a dilute compound solution (8−9 μM) in the same buffer and scanning the UV−vis spectrum after each addition. Titrations were stopped when no shift to the lower energy range of the maximum absorbance wavelength in the spectrum was detected between additions. These absorbance values were converted to v (moles of bound compound/moles of DNA nucleotides) and c (free ligand concentrations) using the free and bound molar extinction coefficients for the analyzed compound. Experimental data in the 0.2−0.8 fraction bound range (results outside this range are subject to large systematic errors due to experimental errors in determining molar extinction coefficients)[53] were plotted using the Scatchards method and analyzed by the extended neighbor exclusion model of McGhee and von Hippel.[31] A nonlinear least-squares fitting procedure[54] was used to derive the binding parameters (K, n) from the following equation:

$$v/c = K(1 - nv)\,[(1 - nv)/(1 - (n - 1)v)]^{n-1}$$

Viscometric Measurements. Samples of DNA (3 mg/mL) in a phosphate buffer 0.01 M (pH = 6.9) were prepared and sonicated as described by Davidson.[55] The average molecular weight of this DNA after sonication was determined to be 3.7 × 10^5 D by viscometric analysis, as described by Eigner and Doty.[56] The purified sonicated DNA sample displayed an A_{260}/A_{280} ratio of between 1.88 and 1.93 and a total hyperchromicity at 260 nm of 30%.

The viscometric titrations were performed in a capillary viscometer at 25 ± 0.01 °C. The buffer used was 50 mM Tris, at pH = 7.56 with 15 mM NaCl. At least four flow times were used to calculate the average relative viscosity for each DNA solution. Reduced specific viscosity was calculated by the method of Cohen and Eisenberg.[34] For each compound 2−4 experiments were carried out. The value of L/L_0 was plotted for each experiment as a function of r. The lines passing through the experimental points were fitted by the least-squares method and forced to pass through the origin.

***In Vitro* Cytotoxicity.** Cytotoxicity in human cell line HT-29 was carried out as decribed in ref 57.

Acknowledgment. We thank Dr. José Gallego for his help at the initial stages of the modeling work and Dr. Miguel Fernandez Braña (Knoll S. A.) for biological activity determinations. Financial support from the Comisión Interministerial de Ciencia y Tecnología (CICYT, project SAF-0280) and studentships (A.M. and B.P.T) from the Ministerio de Educación y Ciencia are gratefully acknowledged.

JO960266H

(53) Deranleau, D. A. *J. Am. Chem. Soc.* **1969**, *91*, 4044.
(54) Hintze, J. L. BMDP Statistical Sofware Inc., Los Angeles, CA, 1991.
(55) Davidson, M. W.; Griggs, B. G.; Boykin, D. W.; Wilson, W. D. *J. Med. Chem.* **1977**, *20*, 1117.
(56) Eigner, J.; Doty, P. *J. Mol. Biol.* **1965**, *12*, 549
(57) Bousquet, P. F.; Fernandez Braña, M.; Conlon, D.; Fitzgerald, K. M.; Perron, D.; Cocchiaro, C.; Miller, R.; Moran, M.; George, J.; Qiann, X.-D, Keilhauer, G.; Romerdahl, C. A. *Cancer Res.* **1995**, *55*, 1176.