inhibition of the RAS. For oral testing, 8c was crushed with a mortar and pestle, suspended in 0.1 M citric acid/H_2O, and given to two animals at dose of 50 mg/kg. Blood samples were collected at -30, -5, 15, 30, and 60 min, and every hour thereafter for 6 h. Averaged results are given in Figure 6. A standard was given at the end of the experiment as in the intravenous testing.

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Registry No. **3a**, 129941-26-0; **3c**, 122662-97-9; **3bd** (epimer 1), 129941-27-1; **3bd** (epimer 2), 129941-28-2; **4a**, 129941-29-3; **4c**, 123350-52-7; **4bd** (epimer 1), 129941-30-6; **4bd** (epimer 2), 129941-31-7; **5d**, 129941-34-0; **5abc** (diastereomer 1), 129896-98-6; **5abc** (diastereomer 2), 129941-32-8; **5abc** (diastereomer 3), 129941-33-9; **6ac** (epimer 1), 129896-99-7; **7ac** (epimer 2), 129897-01-4; **6bd** (epimer 1), 129897-00-3; **6bd** (epimer 2), 129897-02-5; **7ac** (epimer 1), 129897-03-6; **7ac** (epimer 2), 129897-05-8; **7bd** (epimer 1), 129897-04-7; **7bd** (epimer 2), 129897-06-9; **8a**, 129897-07-0; **8c**, 129941-36-2; **8bd** (epimer 1), 129941-35-1; **8bd** (epimer 2), 129941-37-3; **9a**, 130007-84-0; **9b**, 123350-46-9; **10**, 123350-47-0; (5*S*)-11, 122663-19-8; (5*R*)-11, 122663-22-3; (5*S*)-12, 122663-18-7; (5*R*)-12, 122662-87-7; **13a**, 122662-89-9; **13b**, 122663-21-2; **14a**, 119715-71-8; **14a** (R = H), 5165-28-6; **14b**, 129896-97-5; **15**, 103740-50-7; **16**, 122662-94-6; **17**, 129896-88-4; **18**, 129941-24-8; **19**, 129896-89-5; **20**, 129896-90-8; **21**, 129896-91-9; **22**, 129896-92-0; **23**, 129896-93-1; **24**, 129896-94-2; **25**, 122663-39-2; **26**, 122662-80-0; **27**, 122662-84-4; **28**, 122742-12-5; **29**, 122742-13-6; **30**, 122662-77-5; **31**, 122662-83-3; **32**, 122662-78-6; **33**, 122662-82-2; **34**, 129896-95-3; **35**, 129896-96-4; **36**, 122662-76-4; **37**, 122662-79-7; **638**, 122662-81-1; **39**, 122663-51-8; **40**·2TFA, 129941-25-9; H-D-pGlu-OH, 4042-36-8; H-pGlu-OH, 98-79-3; Me-pGlu-OMe, 42435-88-1; Me-D-pGlu-OMe, 122742-14-7; Boc-His(DNP)-OH, 25024-53-7; Boc-Phe-OH, 13734-34-4; 1-(2-hydroxyethyl)-2-pyrrolidinone, 3445-11-2; 1-(3-aminopropyl)-2-pyrrolidinone, 7663-77-6; 1-[3-[(benzyloxycarbonyl)amino]-propyl]-2-pyrrolidinone, 122662-93-5; *N*-methyl-2-pyrrolidinone, 872-50-4; *N*-methyl-2-piperidinone, 931-20-4; perhydro-*N*-methyl-2-azepinone, 2556-73-2; renin, 9015-94-5; cathepsin D, 9025-26-7; cathepsin E, 110910-42-4; *N*-Boc-L-cyclohexylalaninal, 98105-42-1.

Supplementary Material Available: Tables of atomic positional parameters, temperature parameters, and interatomic distances and angles for **3c**, **5d**, and **36** and coordinates for the model of human renin in Protein Data Bank format are available (36 pages). Ordering information is given on any current masthead page.

Syntheses and in Vitro Evaluation of Water-Soluble "Cationic Metalloporphyrin-Ellipticine" Molecules Having a High Affinity for DNA

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The synthesis of hybrid "cationic metalloporphyrin-intercalator" molecules is reported. These molecules are based on 9-methoxyellipticine as intercalator and tris-(4-*N*-methylpyridiniumyl)metalloporphyrins having a 4-aminophenyl or a 4-hydroxyphenyl group for the attachment of the linker. The effect of the length of linker (7-13 bonds), the chemical nature of the linking group (with a carboxamido or an ether function), the position of amino group between the two parts of hybrid molecules, the number of intercalator moieties (ellipticinium) covalently attached to the metalloporphyrin, and the nature of the central metal atom (Mn, Fe, Zn) on the biological activity of these hybrid molecules were studied. In addition, these molecules have a high affinity for double-stranded DNA (affinity constant of hybrid molecule **9Mn,Me** = 2.3×10^9 M^{-1} for poly[d(A-T)] and 2.8×10^8 M^{-1} for poly[d(G-C)] and are cytotoxic against murine leukemia cells L1210 in vitro (IC_{50} of **9Mn,Me** = 0.8 μM). Their cytotoxicities are dependent on the nature of central atom. Iron derivatives are less active than manganese analogues and the corresponding zinc derivatives are nearly inactive despite their same affinity for nucleic acids. These highly water-soluble hybrid molecules could be considered as efficient bleomycin models based on a cationic metalloporphyrin.

Introduction

Bleomycin has to be regarded as a paradigm for synthetic DNA cleavers. This antitumoral antibiotic is able to create single- and also double-strand breaks on duplex DNA in association with three cofactors: iron or copper salts, molecular oxygen, and an electron source.[1] The DNA cleavage occurs via the abstraction of an hydrogen atom at a C_4-position of a deoxyribose ring by "activated bleomycin" (see review articles 1a,b for the mechanism of DNA cleavage and refs 1b,f,g for the possible role of bleomycin-iron-oxo species as reactive intermediate). The structure of bleomycin has two domains: one part of the molecule is involved in the DNA binding mode and five nitrogen atoms from the peptidic chain are the chelating atoms of metal salts.[1] All the modeling studies of bleomycin have been used on this structure duality: the association of an intercalating agent to EDTA[2] or a metalloporphyrin.[3]

Since our group is involved in oxidation reactions catalyzed by metalloporphyrins,[4] DNA cleavage by high-va-

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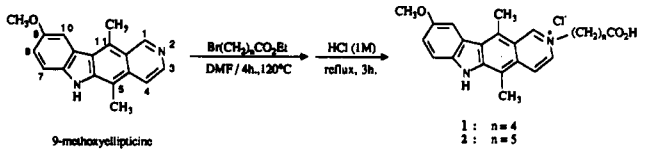
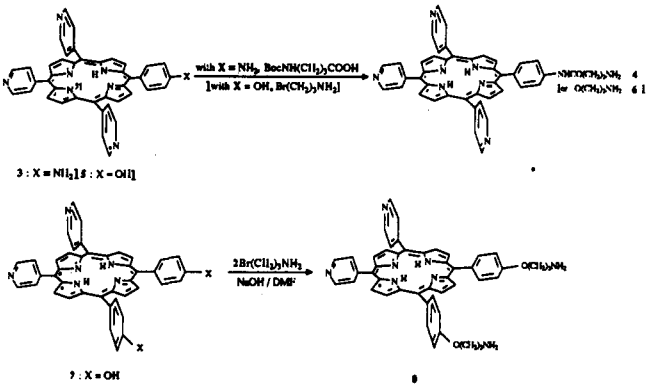
Scheme I. Structures of Ellipticine Derivatives 1 and 2 Used as Intercalating Agents

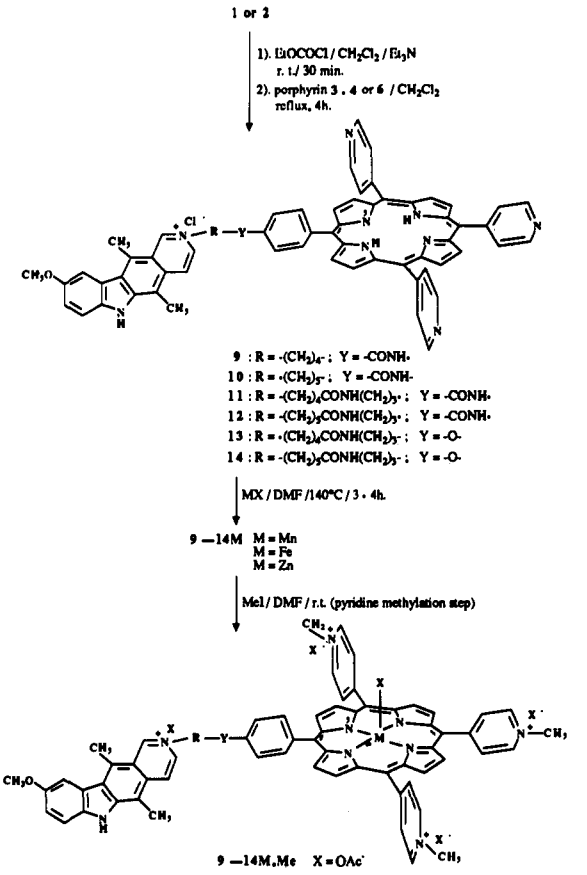


Scheme II. Syntheses of Porphyrin Precursors 4, 6, and 8



lent manganese–oxo species,[5] and the mechanism of action of cytotoxic ellipticine derivatives,[6] we have synthesized hybrid "metalloporphyrin–intercalator" molecules.[7] (The word "hybrid" refers to a molecule made up of chemical molecules having different properties, e.g. a chelating agent and an intercalator. When one of the element is a biomolecule, a protein, or a nucleic acid, the word "bioconjugate" should be used.) However these hybrid molecules are based on a hydrophobic metalloporphyrin moiety which is not able to interact in a close contact with DNA like tetracationic metalloporphyrins.[5,8] Consequently these hybrid molecules do not have high nuclease activity and are not cytotoxic to cells in vitro.[7b] In order to improve the biological activities of these metalloporphyrin–ellipticine hybrid molecules, we have recently developed the preparation of tris-pyridiniumyl water-soluble functionalized metalloporphyrins which exhibit cytotoxicity toward murine leukemia cells L1210 and have nuclease activity on DNA in vitro.[9] Attached to an intercalating agent, these tris-cationic tetrapyrrolic chelating molecules should provide good models for bleomycin. Here we report the chemical syntheses, the cytotoxic properties, and the DNA

Scheme III. Syntheses of Hybrid "Metalloporphyrin–Ellipticine" Molecules 9–14[a]



[a] In the case of zinc complexes, the axial ligand is probably a molecule of water. There are only four X^-, not five as for Fe and Mn complexes (for an X-ray structure of an aquozinc (11) porphyrin complex, see Glick, M. D.; Cohen, G. H.; Hoard, J. L. *J. Am. Chem. Soc.* **1967**, *89*, 1996–1998).

binding constants of these water-soluble hybrid metalloporphyrin–ellipticine molecules (for a preliminary communication, see ref 10).

Results and Discussion

Chemical Syntheses of Hybrid "Metalloporphyrin–Ellipticine" Molecules. The intercalating moiety of these hybrid molecules is 9-methoxyellipticine, which can be easily prepared according to known procedures.[11] The corresponding 9-methoxyellipticinium derivatives having linkers of various length, with a terminal free acid function (**1**, n = 4; **2**, n = 5), were obtained by alkylation of the pyridine nitrogen atom of 9-methoxyellipticine by a ω-bromoalkyl acid ethyl ester in DMF for 4 h at 120 °C, followed by an acid hydrolysis of the ester function under reflux for 3 h, as previously described[7b] (Scheme I).

The chelating group is an unsymmetrical porphyrin functionalized either directly by an amino (**3**) or a hydroxyl group (**5**) or via a linkage finishing with an amino function (**4**, **6**, **8**, Scheme II). 5-(4-Aminophenyl)-10,15,20-tris(4-pyridyl)porphyrin (**3**) was prepared in acid medium according to the classical Adler–Longo method.[12] The reaction yield was optimized after several trials (7%).[9] This porphyrin is actually the precursor of porphyrin **4** attached

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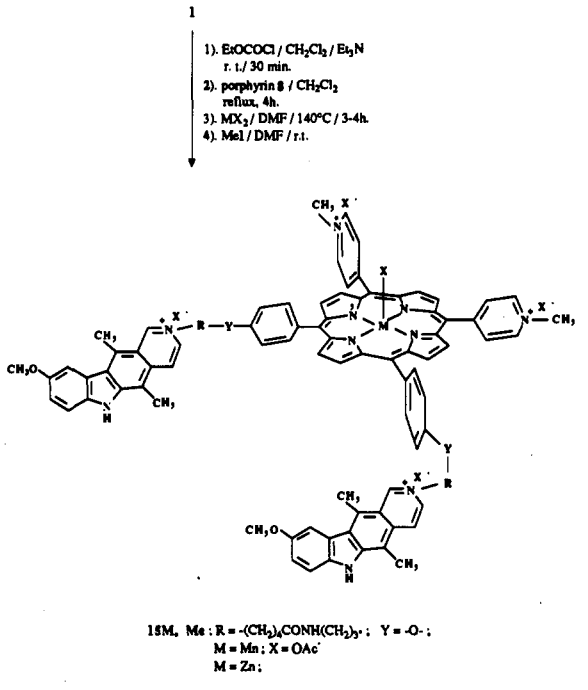
Scheme IV. Syntheses of Metalloporphyrin Complexes 15 Bearing Two Intercalators



15M, Me : R = $-(CH_2)_4CONH(CH_2)_3-$; Y = -O- ;
M = Mn ; X = OAc
M = Zn ;

to an aminomethylene chain by a carboxamido linkage. **4** could be synthesized by the coupling of *N*-Boc-aminobutyric acid and **3** according to the mixed-anhydride method, following by deprotection of amino function in acidic medium (84% yield).[9] Porphyrins **6** and **8**, linked to one or two *n*-propylamines by an ether linkage, were prepared from 5-(4-hydroxyphenyl)-10,15,20-tris(4-pyridyl)porphyrin (**5**) or **7** and 3-bromopropylamine in DMF in the presence of sodium hydroxide (85% and 79% yield, respectively).[9]

All these derivatives are suitable precursors of cationic porphyrins with a function for the anchoring of a vector. In addition, this class of synthetic porphyrins is known to be more resistant to oxidation conditions than natural porphyrins without substituents at meso positions.[4a]

The strategy for the porphyrin–ellipticine linkage synthesis was based on the mixed-anhydride method[13] (Schemes III and IV). Thus, the hybrid molecules were obtained by the coupling of the mixed anhydride formed from **1** and **2** and ethyl chloroformate in CH_2Cl_2 in the presence of triethylamine with the metal-free porphyrin bearing an amino function attached (i) either directly at the para position of phenyl ring (**3**), (ii) by a methylene chain to the p-carboxamido phenyl ring (**4**), (iii) or to the *p*-phenoxy ring (**6** and **8**) of a *meso*-tetraarylporphyrin. The reaction was completed after a 4-h reflux. After purification by preparative thin-layer chromatography (avoiding direct irradiation by light[26]) the pure hybrid molecule was obtained in 45% yield. The field-desorption mass spectrum of **9** ($C_{64}H_{51}N_{10}O_2^+$, OAc^-) gave a peak at 991 ($M^+ - 1$), indicating that the coupling of both parts of the hybrid molecule had occurred.

Because of the photosensitivity of nonmetalated hybrid molecules,[26] the metalation was often performed directly on the crude product without any purification. So, after evaporation of solvent under vacuum, the residue was dissolved in dimethylformamide and the metalation was performed with a large excess of metal salts (10 equiv) in the presence of 2,4,6-collidine at 140 °C for 2–3 h. The completion of the reaction was followed by UV–visible spectrophotometry and by analytical TLC. After purification on dry basic alumina column chromatography, the hybrid "metalloporphyrin–ellipticine" molecules, with manganese, iron, or zinc as the central atom (**9M–15M**), were obtained in 44–79% yield.

In order to prepare the corresponding cationic hybrid molecules, the methylation of pyridine nitrogen atoms of the metalloporphyrin moiety was performed by a large excess of methyl iodide in dimethylformamide at room temperature for 15 h. The cationic hybrid molecules "metalloporphyrin–ellipticine" were obtained in 47–81% yield after the exchange of counterions from iodides to acetates, giving the thoroughly water-soluble compounds (**9M,Me-15M,Me**).

The strong Soret band of these metalloporphyrin derivatives is a characteristic parameter for the recognition of each hybrid molecule. Thus, their UV–visible spectra have, in addition to the band at 315 nm corresponding to the ellipticine moiety, a strong Soret band at 465 nm for manganese porphyrins, at 430 nm for iron derivatives, and at 442 nm for zinc analogues. Since zinc complexes are diamagnetic, their structures could also be determined by ^1H NMR (for details, see the Experimental Section).

The length of linkage between the cationic metalloporphyrin and ellipticinium moiety could be modulated, depending on the length of the functionalized linkers attached to each group. Furthermore, the rigidity of the molecule can be slightly modified when the porphyrin moiety was linked to the tether by a carboxamido or ether function.

Finally, a hybrid molecule "metalloporphyrin–biselliptic ine" **15** was prepared in order to compare the influence of two intercalating groups on the activity of these compounds (for recent articles on DNA interactions with bis-intercalators, see ref 15).

Cytotoxic Activity of Water-Soluble Hybrid "Metalloporphyrin–Ellipticine" Molecules (9M,Me–15M,Me). The cytotoxicities of the hybrid molecules **9M,Me-15M,Me** was tested against murine leukemia cells L1210 in vitro by a procedure already described.[16] The IC_{50} (μM) of hybrid molecules, the concentration which reduces by 50%, after 48 h, the L1210 cell growth as compared to controls, are listed in Table I.

We observed that the cytotoxicity is highly dependent on the nature of the central atom of these metalloporphyrin derivatives. The IC_{50} value of hybrid molecule **9Mn,Me** (n = 4, n being the number of methylene groups) is 0.8 μM, the iron derivative **9Fe,Me** is less toxic (IC_{50} = 1.6 μM) and the zinc analogue has no significant activity (no toxicity is observed for a drug concentration >7.5 μM). The fact that only manganese or iron derivatives are cytotoxic and that their IC_{50} values decrease from manganese to the iron analogue strongly suggests that the biological activities of these cationic hybrid molecules could be related to redox processes or oxygen transfer through the formation of reactive metal–oxo species, as already suggested for bleomycin[1b,f,g] or in DNA cleavage by the hydrogen persulfate/manganese porphyrin system.[5]

Furthermore, the intercalator 9-methoxyellipticine, an antitumor agent by itself,[6b,17] becomes a poor cytotoxic

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Table I. Cytotoxicity against Murine Leukemia L1210 Cells of the Different Hybrid "Metalloporphyrin-Ellipticine" Molecules

compounds	IC_{50},[a] μM	class: no. of bonds between the two parts	class: nature of Y: -NHCO-	class: nature of Y: -O-
Ell-$N^+(CH_2)_4COOH$, 1	>23	0	-	-
Mn^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **9Mn,Me**	0.8 ± 0.2	7	+	-
Fe^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **9Fe,Me**	1.6	7	+	-
Zn^{II}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **9Zn,Me**	*b*	7	+	-
Mn^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **10Mn,Me**	1.4	8	+	-
Mn^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **13Mn,Me**	0.8	11	-	+
Mn^{III}[Por($MePy$)$_2$(Ph-Y-R-Ell)$_2$], **15Mn,Me**	0.7	11	-	+
Mn^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **11Mn,Me**	1.0	12	+	-
Mn^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **14Mn,Me**	1.2	12	-	+
Mn^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **12Mn,Me**	1.2	13	+	-
bleomycin[c]	1.7			

[a] IC_{50} is the concentration able to reduce by 50% in 48 h the growth of L1210 cells as compared to controls. The standard deviation obtained in the case of **9Mn,Me** is based on four independent experiments. For the other reported values, the indicated numbers are the mean value of two or three determinations. [b] No growth inhibition is observed for a drug concentration of 7.5 μM, indicating that the zinc complex has no significant cytotoxic activity in the experimental conditions used for the corresponding manganese or iron derivatives with this type of tumor cells. [c] Bleomycin (Roger-Bellon) was a mixture of 70% A_2 and 30% B_2.

Table II. DNA Affinity Constants of Different Hybrid "Metalloporphyrin-Ellipticine" Molecules

compounds	K_{app}, M^{-1}: poly[d(A-T)]	K_{app}, M^{-1}: poly[d(G-C)]
Ell-$N^+(CH_2)_4COOH$, 1	2.5×10^6	4.9×10^8
Mn^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **9Mn,Me**	2.3×10^9	2.0×10^8
Zn^{II}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **9Zn,Me**	2.0×10^9	1.9×10^8
Mn^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **10Mn,Me**	1.2×10^9	4.0×10^8
Mn^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **13Mn,Me**	6.7×10^9	3.2×10^8[a]
Mn^{III}[Por($MePy$)$_2$(Ph-Y-R-Ell)$_2$], **15Mn,Me**	8.2×10^9	7.0×10^8
Mn^{III}[Por($MePy^+$)$_3$(Ph-Y-R-Ell)], **12Mn,Me**	2.9×10^8	8.2×10^8
Mn^{III}[Por($MePy^+$)$_3$(Ph-N^+Me_3)], **3Mn,Me**	3.4×10^8	1.0×10^8[b]
Mn^{III}[Por($MePy^+$)$_3$(Ph-NHCO-$(CH_2)_3N^+Me_3$)], **4Mn,Me**	5.4×10^8	4.8×10^8
Mn^{III}[Por($MePy^+$)$_3$(PhOH)], **5Mn,Me**	3.8×10^7	1.2×10^7
Mn^{III}[Por($MePy^+$)$_3$(PhO$(CH_2)_3N^+Me_3$)], **6Mn,Me**	8.0×10^8	6.5×10^8

[a] Viscosimetric data on calf thymus DNA indicate that the slope of log η/η_o versus log $(1 + 2r)$ is 2.2 as expected for a monointercalating agent.[24] [b] The value 0.0 found for the slope in viscosimetric data indicates that this manganese porphyrin derivative does not behave as an intercalating agent but binds outside (probably in the minor groove) of polynucleotides by both hydrophobic and electrostatic interactions (for recent review articles on the DNA interaction modes of cationic metalloporphyrin complexes, see ref 8).

agent against L1210 cells (IC_{50} > 23 μM) after its quaternization by a valeric acid group. Finally, since the IC_{50} value of manganese hybrid molecule is close to that of the free [tris(4-*N*-methylpyridiniumyl)(*p*-aminophenyl)-porphyrinato]manganese alone (IC_{50} = 0.6 μM);[9] therefore it is not possible to say if **9Mn,Me** is a prodrug of the metalloporphyrin entity or if the observed cytotoxicity is related to the entire hybrid molecule. But at least the cytotoxicity of the hybrid molecule is not due to the toxicity of the ellipticinium moiety itself after an eventual intracellular hydrolysis of the peptide link.

It should be noted that the IC_{50} values for these "bleomycin-like" molecules are slightly below that of bleomycin itself on the same leukemia cells: 1.7 μM. Despite its unexceptional low activity on leukemia cells in vitro, bleomycin has been successfully developed as antitumor agent in human therapy.[18,1a,b]

In order to study the possible influence of the degrees of freedom between the two moieties of these hybrid molecules on their biological activities, we have determined the IC_{50} values for various compounds by modifying the following parameters: (i) the number of bonds between the intercalator and chelator moieties (length of linker) (7–13 bonds), (ii) the nature of linking group (presence of a carboxamido or an ether function in the chain), and (iii) the position of the amino group between the two parts of hybrid molecule (see Table I). We observed no real significant effects of these three factors on the cytotoxic activity of our hybrid molecules (0.7 μM < IC_{50} < 1.4 μM). The presence of two intercalating groups, covalently attached by ether linkage to porphyrin in the cis position (**15Mn,Me**), did not induce an important increase of cytotoxicity (IC_{50} = 0.7 μM) in comparison with the hybrid molecule having only one ellipticinium group (**13Mn,Me**) (IC_{50} = 0.8 μM).

DNA Affinity Constants of the Hybrid "Cationic Metalloporphyrin-Ellipticine" Molecules. Because these hybrid molecules also have a high nuclease activity on double-stranded DNA in vitro,[19] we have studied their DNA affinity constants. The apparent association constants K_{app} are listed in Table II. The values found, obtained by competition with ethidium bromide,[20] are probably more accurate for the hybrid molecules (which contain an intercalator) than for the metalloporphryin precursors **3Mn,Me–6Mn,Me**. Since these molecules are probably interacting in the minor groove of DNA as the manganese tetrakis(4-*N*-methylpyridiniumyl)porphyrin derivative,[5,21] their K_{app} values might be overestimated because these molecules do not compete for the same binding sites. However, this method has been previously used for minor-groove binders, like netropsin derivatives,[22]

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and provides at least relative rather than absolute binding constants.

The affinity constants of 9-methoxyellipticine-linker 1, determined at a sodium chloride concentration of 10 mM, are equal to 2.5×10^6 and 4.9×10^6 M^{-1} for poly[d(A-T)] and poly[d(G-C)], respectively, while the K_{app} values [tris(4-*N*-methylpyridiniumyl)trimethylaniliniumyl)-porphyrinato]manganese (**3Mn,Me**) alone are 3.4×10^8 M^{-1} for poly[d(A-T)] and 1.0×10^8 M^{-1} for poly[d(G-C)], suggesting a slight preference for A-T sequences compared to G-C's for the metalloporphyrin moiety (such A-T sequence preference for cationic manganese porphyrins have been observed by Dabrowiak et al.[21]).

Under the same conditions, the affinity constants (K_{app}) to polynucleotides (poly[d(A-T)] and poly[d(G-C)]) have been measured for the manganese- and zine-hybrid molecules, **9Mn,Me** and **9Zn,Me**, by using their ability to compete with the binding of ethidium bromide. The K_{app} values are not modulated by the central atom, but a slight preference is observed for poly[d(A-T)] compared to poly[d(G-C)]: for **9Mn,Me**, $K_{app} = 2.3 \times 10^9$ and 2.0×10^8 M^{-1}, respectively. The same difference of 1 order of magnitude in favor of A-T sequences is also observed for the corresponding zinc compound.

K_{app} of the manganese-hybrid molecule **9Mn,Me** has been also determined as a function of the ionic strength.[23] Record plots (log K_{app} vs log [Na^+]) for these data yield straight lines whose slopes are 1.9 ± 0.2 and 1.8 ± 0.1 for poly[d(A-T)] and poly[d(G-C)], respectively. These values, close to 2.0, are in agreement with the involvement of two positive charges in the binding process (ellipticinium derivatives give 1.0).

Viscosimetric studies on calf thymus DNA with **9Mn,Me** indicate that the slope value is 2.2, the expected value for a monointercalating agent.[24] The ellipticine moiety of this hybrid molecule is presumably intercalated between DNA base pairs with the positively charged manganese porphyrin entity strongly interacting outside of the nucleic acid (probably in the minor groove) by both hydrophobic and electrostatic contributions. (Manganese porphyrins are known to be unable to intercalate between DNA base pairs;[8] zinc tetrakis(4-*N*-methylpyridiniumyl)porphyrin is also unable to intercalate, due to the presence of one (or two) water molecule as weak axial ligand.[25] (See Figure 1 for a representation of the possible interaction of a hybrid molecule "metalloporphyrin–ellipticine" with a double-stranded DNA.)

In conclusion, these hybrid "cationic metalloporphyrin–ellipticine" molecules have a high affinity for DNA and are at least as are cytotoxic against murine leukemia cells as bleomycin itself. The fact that the biological activities of these molecules based on a chelating agent are metal dependent suggests that the effect on whole cells might be related to redox processes via a reactive metal–oxo species. These properties (DNA affinity and cytotoxicity) testify that these cationic metalloporphyrin–ellipticine hybrid molecules are efficient bleomycin models. In addition, since their cytotoxic activity is essentially related to the [trispyridiniumyl-porphyrinato]manganese moiety, such part of these molecules can be considered as a useful pharmacophore. This latter entity can be easily linked to various molecules (not only intercalators, but also oligonucleotides, peptides, etc.) for targeting to specific nucleic acids sequences and might have a real future in the design of new DNA-damaging drugs.

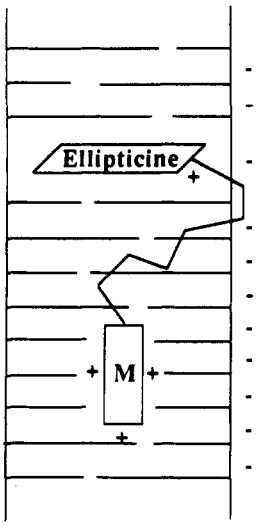

Figure 1. Schematic representation of the possible interaction of a hybrid "metalloporphyrin–ellipticine" molecule with a double-stranded DNA.

Experimental Section

9-Methoxyellipticine was a gift from the Sanofi Co. (Paris, France). Organic chemicals and reagents were obtained from Aldrich and were used as received unless otherwise stated. Anhydrous dichloromethane was prepared by reflux over calcium hydride, followed by distillation, and kept over a 4-Å molecular sieve. Column chromatography was performed on silica gel 60 (70–230 mesh) or basic alumina 90 (70–230 mesh) from Merck. Merck precoated analytical TLC plates (silica gel 60 F_{254} or neutral alumina 150 F_{254}, 0.2 mm) were used for TLC. Ion-exchange resin Amberlite IRN-78 was purchased from Prolabo. Elemental analyses were performed by the Service de Microanalyse du Laboratoire de Chimie de Coordination (CNRS, Toulouse). The following instruments were used for physicochemical data: ^1H NMR, Bruker 250 WM (250 MHz) and AC 200 (200 MHz); UV–visible, Varian-Cary 2300; IR, Perkin-Elmer 983 G; mass spectra, Ribermag R1010 for DCI (NH_3), Varian Mat 311 A for FD. FAB^+ spectra were obtained by the Service Central d'Analyse du CNRS at Lyon.

General Procedure for the Preparation of N^2-(Carboxyalkyl)-9-methoxyellipticinium Chloride. The same method[7b] as previously detailed for N^2-(carboxybutyl)-9-methoxyellipticinium chloride (1) was used for the synthesis of N^2-hexanoic acid analogue **2**.

N^2-(carboxypentyl)-9-methoxyellipticinium chloride (**2**)

$$\underset{17}{HO}\underset{16}{CO}\underset{15}{CH_2}\underset{14}{CH_2}\underset{13}{CH_2}\underset{12}{CH_2}CH_2N^+\text{–Ell}$$

Yield = 95%; UV–vis (MeOH, c = 3.3×10^{-5} M) λ nm (ε, mol^{-1} L cm^{-1}) 440 (2.4×10^3), 382 (6.1×10^3), 313 (5.5×10^4); MS (DCI) *m/e* 391 (M^+); IR (NaCl pellets) ν 1656 cm^{-1} (C=O); ^1H NMR (DMSO-d_6 at 294 K) δ 12.24 (s, 1 H, COOH), 10.20 (s, 1 H, H_1), 8.62 (d, 1 H, J = 7.0 Hz, H_3), 8.56 (d, 1 H, J = 7.0 Hz, H_4), 8.02 (d, 1 H, J = 2.2 Hz, H_{10}), 7.72 (d, 1 H, J = 8.8 Hz, H_7), 7.42 (dd, 1 H, J = 8.8 Hz, J = 2.2 Hz, H_8), 4.81 (t, 2 H, J = 7.2 Hz, $(CH_2)_{12}$), 4.05 (s, 3 H, MeO), 3.44 (s, 3 H, Me_{11}), 2.95 (s, 3 H, Me_5), 2.36 (t, 2 H, J = 7.0 Hz, $(CH_2)_{16}$), 2.13 (m, 2 H, $(CH_2)_{13}$), 1.70 (m, 2 H, $(CH_2)_{15}$), 1.47 (m, 2 H, $(CH_2)_{14}$).

Preparation of Functionalized Porphyrins. The 5-(4-aminophenyl)-10,15,20-tris(4-pyridyl)porphyrin (**3**), 5-[4-[(ami-

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(25) For an example of a zinc porphyrin derivative with an axial water molecule (d_{Zn-O} = 2.20 Å), see: Glick, M. D.; Cohen, G. H.; Hoard, J. L. *J. Am. Chem. Soc.* **1967**, *89*, 1996.
(26) The nonmetalated porphyrins are photoactivable molecules and the exposure of their solutions to light for hours leads to an important degradation of the hybrid molecules.[14]

nobutyryl)amino]phenyl]-10,15,20-tris(4-pyridyl)porphyrin (4), 5-[4-[(3-aminopropyl)oxy]phenyl]-10,15,20-tris(4-pyridyl)-porphyrin (6), and 5,10-[4-[(3-aminopropyl)oxy]phenyl]-15,20-bis(4-pyridyl)porphyrin (8) were prepared according to the method already described.[9]

General Procedure for the Preparation of Hybrid "Metalloporphyrin-Ellipticine" Molecules First Step. An excess of ethyl chloroformate (0.35 mmol, 10 equiv) was added dropwise to a mixture of acid 1 or 2 (0.175 mmol, 5 equiv) and triethylamine (0.35 mmol, 10 equiv) in dry dichloromethane (3 mL). The mixture was stirred at room temperature for 0.5 h then evaporated to dryness under vacuum. The residue was taken up in anhydrous dichloromethane (3 mL). Triethylamine (0.35 mmol) was added followed by porphyrin 3, 4, 6, or 8 (0.035 mmol). The mixture was heated under reflux for 4 h. The progress of the reaction was followed by TLC. The mixture was allowed to cool to room temperature then the solvent was evaporated to dryness under vacuum. The residue, taken up in dichloromethane, was purified on a silica plate (eluent: $EtOH/CH_2Cl_2$ 20/80) in the dark. The product was dissolved in methanol and acetic acid. The filtrate was evaporated to dryness, washed with distilled water, and then dried in a vacuum.

Example: 5-[4-[[4-(9-methoxy-N^2-ellipticinium)butyl]-carboxamido]phenyl]-10,15,20-tris-(4-pyridyl)porphyrin Chloride (9). The carbon atoms of the arm were numbered from 12 to 16, starting from the nitrogen atom 2 of the 9-methoxyellipticine as follows:

Porphyrin-Ph-NHCO$CH_2CH_2CH_2CH_2N^+$-Ell
16 15 14 13 12

Yield = 45%; MS (FD) m/e 991 ($M^+ - 1$); ^1H NMR (DMSO-d_6 at 303 K) δ 10.32 (s, 1 H, H_1), 9.16 (d, 6 H, J = 5.3 Hz, 2,6-pyridine), 9.01 (m, 8 H, β-pyrrole), 8.73 (m, 1 H, H_3), 8.67 (m, 1 H, H_4), 8.38 (d, 6 H, J = 5.3 Hz, 3,5-pyridine), 8.21 (m, 4 H, 4-aminophenyl), 8.09 (br s, 1 H, H_{10}), 7.76 (d, 1 H, J = 8.7 Hz, H_7), 7.44 (br d, 1 H, J = 8.7 Hz, H_8), 4.96 (m, 2 H, $(CH_2)_{12}$), 4.02 (s, 3 H, CH_3O), 3.01 (s, 3 H, Me_5), 2.34 (m, 2 H, $(CH_2)_{15}$), 2.10 (m, 4 H, $(CH_2)_{13\text{ and }14}$), -2.90 (s, 2 H, NH pyrrole), Me_{11} group was masked (water), COOH and NH groups exchanged with residual water.

Second Step. The metalation of the hybrid molecules can take place without purification of the latter. After evaporation of solvent, the residue was dissolved in dry DMF (3 mL). 2,4,6-Collidine (0.385 mmol, 11 equiv) was added and the solution was warmed to 100 °C. An excess of manganese, iron, or zinc salt (0.385 mmol, 11 equiv) ($Mn(CH_3COO)_2 \cdot 4H_2O$, $FeCl_2 \cdot 4H_2O$ or anhydrous $Fe(CH_3COO)_2$, $Zn(CH_3COO)_2 \cdot 2H_2O$) was added to the solution. The mixture was heated at 140 °C for 3 h. The completion of the reaction was followed spectrophotometrically and by analytical TLC. The reaction mixture was then cooled to room temperature. After evaporation of solvent under vacuum, the residue was washed with distilled water then purified on a dry basic alumina column (eluent: CH_2Cl_2 followed by CH_2Cl_2/MeOH).

The metalated hybrid molecules thus obtained were taken up in 3 mL of anhydrous DMF. Methyl iodide (10 equiv) was added to the solution and the mixture was stirred overnight at room temperature. The solvent was evaporated to dryness and the residue taken up in methanol. The addition of ion-exchange resin (Amberlite IRN 78) in acetate form to this solution, followed by stirring for 3 h at room temperature, led to the corresponding acetate after filtration. The filtrate was evaporated to dryness, washed with dichloromethane, and then precipitated from a methanol/acetone mixture (yield = 60–80%).

9Me,I$^-$: yield = 76%; UV-vis (MeOH/AcOH 90/10, $c = 1.0 \times 10^{-5}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 649 (3.4×10^3), 590 (3.0×10^3), 546 (5.3×10^3), 510 (1.2×10^3), 413 (2.2×10^5, Soret band), 315 (1.4×10^5).

9Me,AcO$^-$: yield = 76%; UV-vis (MeOH, c = 4.7×10^{-5} M) λ nm (ϵ, mol^{-1} L cm^{-1}) 652 (1.7×10^3), 590 (1.5×10^3), 545 (2.3×10^3), 512 (5.3×10^3), 415 (7.7×10^4, Soret band), 318 (2.7×10^4); MS (FAB^+ in HCl) m/e 1091; ^1H NMR (DMSO-d_6 at 296 K) δ 10.32 (s, 1 H, H_1), 9.16 (d, 6 H, J = 5.1 Hz, 2,6-pyridine), 9.00 (m, 8 H, β-pyrrole), 8.73 (m, 1 H, H_3), 8.61 (m, 1 H, H_4), 8.37 (d, 6 H, J = 5.1 Hz, 3,5-pyridine), 8.24 (s, 4 H, 4-aminophenyl), 8.03 (s, 1 H, H_{10}), 7.73 (d, 1 H, J = 8.7 Hz, H_7), 7.42 (dd, 1 H, J = 8.7, 2.4 Hz, H_8), 4.98 (m, 2 H, $(CH_2)_{12}$), 4.80 (br s, 9 H, NCH_3), 4.01 (s, 3 H, CH_3O), 3.70 (s, 3 H, Me_{11}), 2.98 (s, 3 H, Me_5), 2.33 (m, 2 H, $CH_2)_{15}$), 2.13 (m, 4 H, $(CH_2)_{13\text{ and }14}$), 2.03 (s, 3 H, CH_3COO), 2.91 (s, 2 H, NH pyrrole).

Hybrid "Metalloporphyrin-Ellipticine" Molecules Resulting (a) from the Coupling of Porphyrin 3 with Acid Derivative 1 (Length of Linkage: 7 Bonds). **9Mn,Me**: yield = 83%; UV-vis (H_2O, c = 1.1×10^{-5} M) λ (ϵ, mol^{-1} L cm^{-1}) nm 596 (3.6×10^3), 562 (6.8×10^3), 464 (6.8×10^4, Soret band), 313 (5.3×10^4); MS (FAB^+) m/e 1182.

9Fe,Me: yield = 56%, UV-vis (H_2O, $c = 1.3 \times 10^{-5}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 430 (2.7×10^4, Soret band), 315 (2.4×10^4); MS (FAB^+) m/e 1183.

9Zn,Me: yield = 81%; UV-vis (H_2O, $c = 1.1 \times 10^{-5}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 612 (8.0×10^3), 568 (1.5×10^4), 440 (1.5×10^5, Soret band), 312 (9.1×10^4), MS (FAB^+) m/e 1151.

Since zinc hybrid molecules are diamagnetic, their ^1H NMR spectra can be described. **9Zn,Me**: ^1H NMR (DMSO-d_6 at 303 K) δ 10.24 (s, 1 H, H_1), 9.54 (d, 6 H, J = 5.2 Hz, 2,6-pyridine), 9.00 (m, 8 H, β-pyrrole), 8.78 (d, 1 H, J = 7.4 Hz, H_3), 8.56 (m, 3 H, H_4 and 2,6-(4-aminophenyl)), 8.20 (m, 6 H, 3,5-pyridine), 8.09 (br s, 1 H, H_{10}), 7.77 (2 d, 3 H, J = 8.7 Hz, H_7 and 3,5-(4-aminophenyl)), 7.45 (d, 1 H, J = 8.7 Hz, H_8), 5.02 (m, 2 H, $(CH_2)_{12}$), 4.83 (br s, 9 H, N-Me), 4.04 (s, 3 H, OCH_3), 3.03 (s, 3 H, Me_5), 2.34 (m, 2 H, $(CH_2)_{15}$), 2.12 (m, 4 H, $(CH_2)_{13\text{ and }14}$), 2.02 (s, 3 H, CH_3COO^-); Me_{11} group was masked (water) and NH groups were exchanged with residual water.

(b) From the Coupling of Porphyrin 3 with Acid 2 (Length of Linkage: 8 Bonds).

Porphyrin-Ph-NHCO$CH_2CH_2CH_2CH_2CH_2N^+$-Ell
17 16 15 14 13 12

10Zn: yield = 79%; UV-vis (MeOH, c = 3.0×10^{-6} M) λ nm (ϵ, mol^{-1} L cm^{-1}) 594 (5.3×10^3), 544 (1.6×10^4), 420 (4.22×10^5, Soret band), 314 (6.3×10^4); ^1H NMR (DMSO-d_6 at 303 K) δ 10.30 (s, 1 H, H_1), 9.12 (d, 6 H, J = 5.0 Hz, 2,6-pyridine), 8.94 (m, 8 H, β-pyrrole), 8.72 (d, 1 H, J = 7.1 Hz, H_3), 8.62 (d, 1 H, J = 7.0 Hz, H_4), 8.32 (d, 6 H, J = 5.0 Hz, 3,5-pyridine), 8.17 (m, 4 H, 4-aminophenyl), 8.07 (d, 1 H, J = 2.2 Hz, H_{10}), 7.70 (d, 1 H, J = 8.7 Hz, H_7), 7.39 (d, 1 H, J = 8.7, 2.2 Hz, H_8), 4.93 (m, 2 H, $(CH_2)_{12}$), 3.98 (s, 3 H, MeO), 2.97 (s, 3 H, Me_5), 2.29 (m, 2 H, $(CH_2)_{16}$), 1.95 (m, 4 H, $(CH_2)_{13\text{ and }15}$), 1.65 (m, 2 H, $(CH_2)_{14}$); Me_{11} group was masked (water), and NH groups were exchanged with residual water.

10Mn,Me: yield = 76%; UV-vis (H_2O, $c = 1.86 \times 10^{-5}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 600 (4.3×10^3), 564 (7.4×10^3), 464 (6.5×10^4, Soret band), 314 (5.6×10^4).

(c) From the Coupling of Porphyrin 4 with Acid 1 (Length of Linkage: 12 Bonds).

Por-Ph-NHCO$CH_2CH_2CH_2$NHCO$CH_2CH_2CH_2CH_2N^+$-Ell
20 19 18 17 16 15 14 13 12

11Zn: yield = 60%; UV-vis (MeOH, $c = 1.1 \times 10^{-5}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 598 (3.6×10^3), 556 (1.3×10^4), 421 (2.8×10^5, Soret band), 315 (5.7×10^4); MS (FAB^+) m/e 1140; ^1H NMR (DMSO-d_6 at 303 K) δ 10.21 (s, 1 H, H_1), 9.10 (d, 6 H, J = 5.6 Hz, 2,6-pyridine), 8.98 (d, 2 H, J = 4.7 Hz, β-pyrrole), 8.93 (s, 4 H, β-pyrrole), 8.89 (d, 2 H, J = 4.7 Hz, β-pyrrole), 8.63 (d, 1 H, J = 7.1 Hz, H_3), 8.52 (d, 1 H, J = 7.1 Hz, H_4), 8.31 (d, 6 H, J = 5.6 Hz, 3,5-pyridine), 8.19 (br s, 4 H, 4-aminophenyl), 7.96 (d, 1 H, J = 2.2 Hz, H_{10}), 7.68 (d, 1 H, J = 8.8 Hz, H_7), 8.38 (dd, 1 H, J = 8.8, 2.2 Hz, H_8), 4.87 (t, 2 H, J = 6.8 Hz, $(CH_2)_{12}$), 3.99 (s, 3 H, MeO), 3.39 (s, 3 H, Me_{11}), 2.90 (s, 3 H, Me_5), 2.39 (t, 2 H, J = 6.9 Hz, $(CH_2)_{19}$), 2.18 (m, 4 H, $(CH_2)_{13\text{ and }15}$), 1.99 (t, 2 H, J = 6.9 Hz, $(CH_2)_{17}$), 1.76 (m, 4 H, $(CH_2)_{14\text{ and }18}$); NH groups exchange with residual water.

11Mn,Me: yield = 71%, UV-vis (H_2O, $c = 5.3 \times 10^{-5}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 602 (3.4×10^3), 565 (6.8×10^3), 465 (7.2×10^4, Soret band), 315 (5.0×10^4).

(d) From the Coupling of Porphyrin 4 with Acid 2 (Length of Linkage: 13 Bonds).

Por-Ph-NHCO$CH_2CH_2CH_2$NHCO$CH_2CH_2CH_2CH_2CH_2N^+$-Ell
21 20 19 18 17 16 15 14 13 12

12Zn: yield = 47%; UV-vis (DMF, c = 2.6×10^{-6} M) λ nm (ϵ, mol^{-1} L cm^{-1}) 598 (5.5×10^3), 556 (1.6×10^4), 425 (4.2×10^5,

Soret band), 316 (6.0×10^4); MS (FAB$^+$) m/e 1154 (M^+); ^{1}H NMR (DMSO-d_6 at 294 K) δ 10.19 (s, 1 H, H_1), 9.12 (d, 6 H, J = 5.8 Hz, 2,6-pyridine), 8.99 (d, 2 H, J = 4.7 Hz, β-pyrrole); 8.94 (s, 4 H, β-pyrrole), 8.91 (d, 2 H, J = 4.7 Hz, β-pyrrole), 8.62 (d, 1 H, J = 6.9 Hz, H_3), 8.55 (d, 1 H, J = 6.9 Hz, H_4), 8.33 (d, 6 H, J = 5.8 Hz, 3,5-pyridine), 8.22 (d, 2 H, J = 8.6 Hz, 2,6-(4-aminophenyl)), 8.15 (d, 2 H, J = 8.6 Hz, 3,5-(4-aminophenyl), 8.03 (d, 1 H, J = 2.3 Hz, H_{10}), 7.72 (d, 1 H, J = 8.7 Hz, H_7), 7.42 (dd, 1 H, J = 8.7, 2.3 Hz, H_8), 4.84 (m, 2 H, $(CH_2)_{12}$), 4.03 (s, 3 H, MeO), 3.39 (s, 3 H, Me_{11}), 2.90 (s, 3 H, Me_5), 2.28 (m, 4 H, $(CH_2)_{16 \text{ and } 20}$), 2.17 (m, 2 H, $(CH_2)_{18}$), 1.94 (m, 2 H, $(CH_2)_{13}$), 1.77 (m, 4 H, $(CH_2)_{15 \text{ and } 19}$), 1.50 (m, 2 H, $(CH_2)_{14}$); NH groups exchange with residual water.

12Mn,Me: yield = 53%; UV-vis (H_2O, $c = 8.9 \times 10^{-6}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 598 (3.4×10^3), 562 (7.3×10^3), 464 (6.6×10^4, Soret band), 314 (4.6×10^4).

(e) From the Coupling of Porphyrin 6 with Acid 1 (Length of Linkage: 11 Bonds).

Porphyrin-Ph-$OCH_2CH_2CH_2NHCOCH_2CH_2CH_2CH_2N^+$-Ell
19 18 17 16 15 14 13 12

13Zn: yield = 66%; UV-vis (MeOH, $c = 1.6 \times 10^{-6}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 596 (6.3×10^3), 554 (2.2×10^4), 420 (5.0×10^5, Soret band), 314 (7.5×10^4); MS (FAB$^+$) m/e 1114 (M^+); ^{1}H NMR (DMSO-d_6 at 303 K) δ 10.26 (s, 1 H, H_1), 9.11 (d, 6 H, J = 4.6 Hz, 2,6-pyridine), 8.93 (m, 8 H, β-pyrrole), 8.66 (d, 1 H, J = 6.7 Hz, H_3), 8.58 (d, 1 H, J = 6.7 Hz, H_4), 8.32 (d, 6 H, J = 4.6 Hz, 3,5-pyridine), 8.15 (d, 2 H, J = 8.4 Hz, 2,6-(4-phenoxy)), 8.01 (br s, 1 H, H_{10}), 7.65 (d, 1 H, J = 8.6 Hz, H_7), 7.43 (d, 2 H, J = 8.4 Hz, 3,5-(4-phenoxy)), 7.35 (d, 1 H, J = 8.6 Hz, H_8), 4.88 (m, 2 H, $(CH_2)_{12}$), 4.39 (m, 2 H, $(CH_2)_{19}$), 4.00 (s, 3 H, MeO), 2.91 (s, 3 H, Me_5), 2.39 (m, 2 H, $(CH_2)_{15}$), 2.17 (m, 4 H, $(CH_2)_{13 \text{ and } 17}$), 1.76 (m, 4 H, $(CH_2)_{14 \text{ and } 18}$); Me_{11} group was masked (water), and NH groups were exchanged with residual water.

13Mn,Me: yield = 60%; UV-vis (H_2O, $c = 1.1 \times 10^{-5}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 596 (1.9×10^3), 564 (3.6×10^3), 464 (3.5×10^4, Soret band), 314 (2.3×10^4).

(f) From the Coupling of Porphyrin 6 with Acid 2 (Length of Linkage: 12 Bonds).

Por-Ph-$OCH_2CH_2CH_2NHCOCH_2CH_2CH_2CH_2CH_2N^+$-Ell
20 19 18 17 16 15 14 13 12

14Zn: yield = 66%; UV-vis (MeOH, $c = 8.7 \times 10^{-6}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 600 (3.7×10^3), 558 (1.2×10^4), 422 (2.4×10^5, Soret band), 316 (7.6×10^4), ^{1}H NMR (DMSO-d_6 at 303 K) δ 10.09 (br s, 1 H, H_1), 9.10 (d, 6 H, J = 4.3 Hz, 2,6-pyridine), 8.97 (d, 2 H, J = 4.7 Hz, β-pyrrole), 8.91 (s, 4 H, β-pyrrole), 8.89 (d, 2 H, J = 4.7 Hz, β-pyrrole), 8.56 (d, 1 H, J = 6.1 Hz, H_3), 8.43 (d, 1 H, J = 6.1 Hz, H_4), 8.31 (d, 6 H, J = 4.3 Hz, 3,5-pyridine), 8.19 (d, 2 H, J = 8.4 Hz, 2,6-(4-phenoxy)), 7.89 (br s, 1 H, H_{10}), 7.67 (d, 1 H, J = 8.5 Hz, H_7), 7.42 (d, 2 H, J = 8.4 Hz, 3,5-(4-phenoxy)), 7.37 (d, 1 H, J = 8.5 Hz, H_8), 4.79 (m, 2 H, $(CH_2)_{12}$), 4.34 (m, 2 H, $(CH_2)_{20}$), 3.98 (s 3 H, MeO), 3.27 (s, 3 H, Me_{11}), 3.01 (s, 3 H, Me_5), 2.31 (t, 2 H, J = 6.6 Hz, $(CH_2)_{16}$), 2.13 (m, 4 H, $(CH_2)_{13 \text{ and } 18}$), 1.78 (m, 4 H, $(CH_2)_{15 \text{ and } 19}$), 1.50 (m, 2 H, $(CH_2)_{14}$); NH groups exchange with residual water.

14Mn,Me: yield = 60%; UV-vis (H_2O, $c = 5.3 \times 10^{-6}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 620 (5.2×10^3), 674 (8.5×10^3), 466 (7.4×10^4, Soret band), 315 (7.5×10^4).

(g) From the Coupling of Porphyrin 8 with Acid 1 (Length of Linkage: 11 Bonds).

cis-Por-[Ph-$OCH_2CH_2CH_2NHCOCH_2CH_2CH_2CH_2N^+$-Ell]$_2$
19 18 17 16 15 14 13 12

15Zn: yield = 44%; UV-vis (MeOH/DMF 98/2, c = 4.3×10^{-6} M) λ nm (ϵ, mol^{-1} L cm^{-1}) 600 (4.7×10^3), 556 (1.4×10^4), 423 (3.7×10^5, Soret band), 316 (8.6×10^4); MS (FAB$^+$) m/e 1545 (M^+); ^{1}H NMR (DMSO-d_6 at 298 K) δ 10.26 (s, 1 H, H_1), 10.21 (s, 1 H, H_1), 9.12 (d, 4 H, J = 5.0 Hz, 2,6-pyridine), 8.92 (m, 8 H, β-pyrrole), 8.60 (m, 4 H, H_3 and H_4), 8.31 (d, 4 H, J = 5.0 Hz, 3,5-pyridine), 8.16 (d, 4 H, J = 8.6 Hz, 2,6-(4-phenoxy)), 8.03 (br s, 1 H, H_{10}), 7.93 (br s, 1 H, H_{10}), 7.67 (d, 1 H, J = 8.8 Hz, H_7), 7.62 (d, 1 H, J = 8.8 Hz, H_7), 7.42 (d, 4 H, J = 8.6 Hz, 3,5-(4-phenoxy)), 7.35 (d, 1 H, J = 8.8 Hz, H_8), 7.31 (d 1 H, J = 8.8 Hz, H_8), 4.87 (br s, 4 H, $(CH_2)_{12}$), 4.38 (br s, 4 H, $(CH_2)_{19}$), 4.01 (s, 3 H, MeO), 3.99 (s, 3 H, MeO), 3.45 (s, 3 H, Me_{11}), 3.44 (s, 3 H, Me_{11}), 2.92 (s, 3 H, Me_5), 2.87 (s, 3 H, Me_5), 2.39 (m, 4 H, $(CH_2)_{15}$), 2.16 (m, 8 H, $(CH_2)_{13 \text{ and } 17}$), 1.76 (m, 8 H, $(CH_2)_{14 \text{ and } 18}$); NH groups exchange with residual water.

15Mn,Me: yield = 47%; UV-vis (H_2O, $c = 2.15 \times 10^{-5}$ M) λ nm (ϵ, mol^{-1} L cm^{-1}) 600 (4.9×10^3), 566 (7.4×10^3), 466 (6.6×10^4, Soret band), 313 (5.3×10^4); MS (FAB$^+$) m/e 1248.

Determination of Cytotoxicity of Metalloporphyrin Derivatives. The cytotoxicity has been tested in vitro on murine leukemia L1210 cells according to a previously reported method.[16] The metalloporphyrin derivatives were dissolved in water. The inhibitory efficiency against cell multiplication is expressed in terms of IC_{50}, which represents the drug concentration that reduces the rate of cell multiplication by 50% as compared to controls.

Determination of DNA Binding Constants of Metalloporphyrin Derivatives. The binding constants K_{app} were determined as described by Baguley et al.[20,23] Under standard conditions, measurements were done at 25 °C in 0.01 M cacodylate buffer at pH 7.0 in 10 mM NaCl solutions with 1.26 μM ethidium bromide (EB) and 1 μM of polynucleotide. Following the fluorescence of bound ethidium bromide in the presence of various concentrations of metalloporphyrin derivatives, the IC_{50} (i.e. the concentration of tested drug required to displace 50% of ethidium bromide from its binding sites) can be accurately estimated from the best fit of data prints obtained through nonlinear-regression procedure. The association constant value $K_{app_{M\text{-}Porp}}$ of the competiting metalloporphyrin derivative M-Porp can therefore be estimated with the following relation:

$$K_{app_{M\text{-}Porp}} = K_{app_{EB}}[\text{M-Porp}]/[\text{EB}]$$

where [M-Porp] and [EB] are, respectively, the concentration of M-Porp and ethidium bromide at the IC_{50} value and $K_{app_{EB}}$ the association constant of ethidium bromide for the polynucleotide used (9.5×10^6 and 9.6×10^6 M^{-1} for poly[d(A-T)] and poly[d-(G-C)], respectively).

The viscosimetric determination of length increase of sonicated calf thymus DNA by some hybrid molecules metalloporphyrin–ellipticine has been done according to ref 24. η and η_0 are the intrinsic viscosity values of sonicated calf thymus DNA in the presence or in the absence of tested drug, and r is the number of molecules bound per nucleotide (see ref 24 for more experimental details on viscosimetric data).

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