Preparation of Tetracationic Metalloporphyrin−Spermine Conjugates

Andreas Jakobs, Jean Bernadou,* and Bernard Meunier*

Laboratoire de Chimie de Coordination du CNRS, 205 route de Narbonne, 31077 Toulouse Cedex 4, France

Received November 6, 1996[⊗]

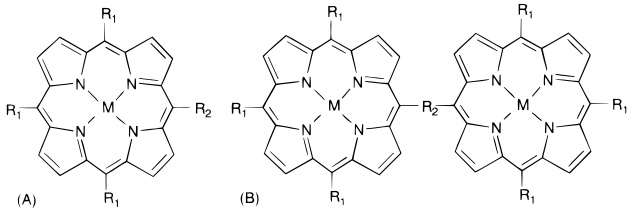
The preparation of tetracationic metalloporphyrin precursors for the attachement to DNA binding molecules is reported. Based on a D_{4h} tetrapyridylporphyrin, such precursors are more accessible than the classical tailored tricationic porphyrin derivatives used as DNA cleavers. *meso*-Tetrapyridylporphyrin was reacted with ethyl bromopentanoate and methyl iodide to afford a monofunctionalized ester derivative of **1** which was hydrolyzed to the corresponding acid **5**. This acid was reacted with spermine and BOP to yield conjugates containing one (**6**) or two (**7**) porphyrin units that were metalated with Mn(II) salts to afford the metalloporphyrins **6**−Mn and **7**−Mn. Furthermore, **6**−Mn was linked to **5** to yield a spermine conjugate **8** containing two porphyrin moieties, one of which was metalated. The DNA cleavage activity of these different metalloporphyrins was studied in the presence of $KHSO_5$ with double-stranded ΦX174 DNA. Conjugate **5**−Mn, the spermine containing compound **6**−Mn, and compound **7**−Mn which contained two porphyrin moieties showed cleavage activities similar to that of the parent DNA cleaver **1**−Mn. The porphyrin conjugate **8** which contained one metalated and one nonmetalated porphyrin had half the efficiency of **1**−Mn. None of the synthesized compounds were able to induce direct double-strand breaks in the experimental conditions used.

Introduction

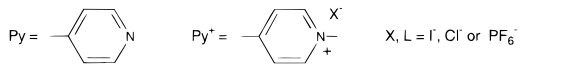
The design of artificial nucleases is a growing research area because major advancements in this field would have important implications as tools in molecular biology and also as potential chemotherapeutic agents in the field of cancer or antiviral therapy. A nuclease activity can be achieved on DNA or RNA either by hydrolysis of the phosphodiester bond or by oxidative cleavage.[1] Site-specific cleavage could be obtained by tethering the DNA cleavers to oligonucleotides in an antisense or an antigene approach.[2]

The water-soluble diaquamanganese(III) *meso*-tetrakis-(1-methylpyridinium-4-yl)porphyrin pentaacetate (**1**−Mn, see Table 1 for structure) activated by $KHSO_5$, an oxygen atom donor, has been shown to be an efficient artificial endonuclease.[3] This porphyrin derivative exhibits a high affinity toward DNA, not only because of the electrostatic interactions of the four positive pyridinium substituents charges with the DNA phosphate backbone[4] but also because of its ability to strongly interact within the minor groove of B-DNA as evidenced by sugar degradation product identification. The active form of this metalloporphyrin-based DNA cleaver is a high-valent metal−oxo species,[5] similar to the activated form of iron−bleomycin[6] or to the perferryl species of cytochrome P-450.[7] We have shown that the high-valent metal−oxo porphyrin complex ("activated form" of the **1**−Mn/$KHSO_5$ system) was able to hydroxylate C−H bonds at C1′ and

Table 1. Structure of the Porphyrin Derivatives 1−8



	M=H_2	M=Mn^{III}-L	R_1	R_2
	1	**1**-Mn	4-Py$^+$Me	4-Py$^+$Me
	2	-	4-Py	4-Py
A	**3**	-	4-Py	4-Py$^+$(CH$_2$)$_4$CO$_2$C$_2$H$_5$
	4	-	4-Py$^+$Me	4-Py$^+$(CH$_2$)$_4$CO$_2$C$_2$H$_5$
	5	**5**-Mn	4-Py$^+$Me	4-Py$^+$(CH$_2$)$_4$CO$_2$H
	6	**6**-Mn	4-Py$^+$Me	4-Py$^+$(CH$_2$)$_4$CONH(CH$_2$)$_3$NH(CH$_2$)$_4$NH(CH$_2$)$_3$NH$_2$ (*spm = spermine*)
B	**7**	**7**-Mn	4-Py$^+$Me	4-Py$^+$(CH$_2$)$_4$COspmCO(CH$_2$)$_4$-4-Py$^+$
	8	(M=H_2, M=Mn^{III}-L)	4-Py$^+$Me	4-Py$^+$(CH$_2$)$_4$COspmCO(CH$_2$)$_4$-4-Py$^+$



* Author to whom correspondence should be addressed. Phone: (33) 5 61 33 31 46. Fax: (33) 5 61 55 30 03. E-mail: bmeunier@lcc-toulouse.fr.

⊗ Abstract published in *Advance ACS Abstracts,* May 1, 1997.

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S0022-3263(96)02083-X CCC: $14.00

C5′ of DNA deoxyriboses through a strong interaction of the cationic compound within the minor groove of B-type DNA.[3b,5b,8]

The aim of the present work was to improve the DNA cleavage efficiency of hybrid "metalloporphyrin−vector" molecules by replacing the usual tricationic metalloporphyrin moiety by a tetracationic porphyrin skeleton, since previous studies showed the influence of the nature of the functionalized aryl group in the *meso* position of the metalloporphyrin precursor and the better interaction with DNA of tetracationic versus tricationic ones.[9] Recent works on tailored metalloporphyrin−vector molecules were based on tricationic manganese porphyrins coupled to the intercalating agent ellipticine[9] or oligonucleotides,[10] and so these conjugates did not make use of the full DNA-interaction potential of the parent tetracationic **1**−Mn DNA cleaver. Moreover, the further development of such hybrid molecules for animal experiments now requires improved methods of synthesis of the metalloporphyrin precursor. In this work, we report the preparation of functionalized tetracationic metalloporphyrin precursors and their coupling with spermine, which is known for its own strong DNA interaction (the four positive charges of spermine at physiological pH are attracted by the negative charges of the sugar phosphate backbone of DNA and also interact within the minor groove of DNA[11]). A further enhancement of DNA interactions should be obtained by coupling of the tetracationic metalloporphyrin−spermine conjugate either with an unmetalated tetracationic porphyrin to improve the DNA affinity by intercalation or with a second metalated porphyrin ligand to introduce the possibility of direct double-strand cleavage.

Results and Discussion

Preparation of the Tetracationic Porphyrin Precursor. Among water-soluble cationic porphyrins bearing a single functional group to tether a vector, only tricationic porphyrins have been synthesized,[12] except for the mention of the synthesis of a tetracationic derivative in one patent.[13] The general procedure to obtain these monofunctionalized porphyrins utilizes a modification of the classical Adler−Longo[14] method by reacting a mixture of two different benzaldehydes (e.g. 4-formylpyridine and 4-hydroxybenzaldehyde or 4-formylbenzoic acid) and pyrrole.[15] As expected from the statistical formation of at least six different porphyrins, this method requires tedious purification procedures and affords the target porphyrins in only 5−7% yield, making this approach not feasible for up-scale preparations. For some monosubstituted hydrophobic porphyrins, a different strategy that consisted in the monofunctionalization of a readily-available symmetrical porphyrin had been employed (e.g. mononitration of *meso*-tetraphenylporphyrin and subsequent reduction to generate an amine function).[16] In a similar way, the monoalkylation of the *meso*-tetrapyridylporphyrin with 3-bromopropionic acid gave only a poor yield in monofunctionalized derivative (not exceeding that of the mixed aldehyde method), mainly because the different *N*-alkylation products had to be separated by chromatography columns followed by several extraction/precipitation steps.[13]

The present report on the synthesis of monofunctionalized tetrapyridylporphyrin derivatives differs from the common "mixed aldehyde" method and introduces the functionalization after the construction of the porphyrin ring. Tetrapyridylporphyrin **2**, obtained by the classical Adler−Longo procedure,[17] was alkylated with an excess of ethyl 5-bromopentanoate in $CHCl_3$/EtOH, leading to a mixture of different *N*-alkylation products, out of which 5-(1-(4-(ethoxycarbonyl)butyl)pyridinium-4-yl)-10,15,20-tripyridylporphyrin bromide **3** was separated by chromatography on a short silica gel column and obtained in a reasonable 33% yield. Contrary to the method of Schmidt and Steffen,[13] the present preparation afforded the ester **3** in a large scale associated with an easy separation of the expected isomer. The remaining three free pyridin substituents of **3** were then quantitatively alkylated by methyl iodide to afford the tetracationic porphyrin **4**. The ester function of **4** was hydrolyzed with aqueous HCl to the corresponding acid **5** in 91% yield. Metalation of **5** with manganese(II) chloride gave **5**−Mn in 75% yield.

Preparation of Porphyrin−Spermine Conjugates. The covalent attachment of spermine to the porphyrin precursor **5** by formation of an amide bond was less obvious than expected for different reasons. Compound **5** with the free carboxylic function was not soluble in most of the solvents used in classical amide synthesis and was only sparingly soluble in DMF even after the exchange of the iodide counterions for the less polar hexafluorophosphate ions. Only water, DMSO, or a mixture DMSO/CH_2Cl_2 seemed to be suitable solvents for this amide bond formation in terms of solubility. Spermine carries four amine functions with pK_a values of 11.50, 10.95, 9.79, and 8.9,[18] and the primary amines have been shown to be about 100 times more nucleophilic than the secondary amines.[19] Some amide bond formations with unprotected spermine have been described; however these methods used *p*-nitrophenyl esters in methanol[20] or acid chlorides,[21] affording a mixture of mono- and bisacylated spermine derivatives. In water, no coupling reaction was observed with water-soluble

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Table 2. Electrospray Mass Spectrometry Data on Cationic Porphyrin Derivatives 3−8[a]

				molecular peak(s)			other peak(s)		
compd	charge	formula	MW	m/z	calcd	obsd	m/z	calcd	obsd
3	1	$C_{47}H_{39}N_8O_2$	747.89	m	747.9	747.4			
				$m + H^+/2$	374.4	374.4			
4	4	$C_{50}H_{48}N_8O_2$	792.99	m/4	198.3	198.2	Por/3	221.3	221.4
				$m + e^-/3$	264.3	264.2	$Por-Me^+/2$	324.4	324.3
				$m - H^+ + e^-/2$	396.0	395.9	$Por-H^+/2$	331.4	331.4
5	4	$C_{48}H_{44}N_8O_2$	764.93	m/4	191.2	191.3	Por/3	221.3	221.3
				$m - H^+/3$	254.6	254.7	$Por-H^+/2$	331.4	331.5
							$m - 2H^+/2$	381.5	381.5
5−Mn	5	$C_{48}H_{42}N_8O_2$	817.85				Mn−Por/3	238.9	238.8
6	4	$C_{58}H_{68}N_{12}O$	949.26	m/4	237.3	237.3	Por/3	221.3	221.3
							$Por-H^+/2$	331.4	331.4
6−Mn	5	$C_{58}H_{66}N_{12}OMn$	1002.18	m/4	250.6	250.7	Mn−Por/3	238.9	239.0
7	8	$C_{106}H_{110}N_{20}O_2$	1696.17	$m - H^+/7$	242.2	241.8	Por/3	221.3	221.3
							$Por-H^+/2$	331.4	331.4
7−Mn	10	$C_{106}H_{106}N_{20}O_2Mn_2$	1802.02				Mn−Por/3	238.9	238.9
8	9	$C_{106}H_{108}N_{20}O_2Mn$	1749.10				Por/3	221.3	221.0
							Mn−Por/3	238.9	238.5

[a] Formulas and MW are calculated without the counterions and, in the case of manganese derivatives, without the extra counterion and the axial ligand if present. Por represents the fragment when the linker is cleaved at its junction with the pyridinium group (see formula A with R_1 = 4-Py^+Me and R_2 = 4-Py in Table 1). The 100% peaks on the spectra were underlined.

coupling reagents,[22] and this low reactivity might be related to the strong internal hydrogen bonding of spermine and/or the hydrolysis of activated esters used in the coupling reaction. In DMSO, due to the high polarity of this reaction medium, the activated esters are often unstable and rearrange or decompose; in our case, a great number of different coupling reagents gave only low yields in coupling products as evidenced by NMR spectra of the reaction medium. Because of the highly charged porphyrin moiety, preparative scale chromatographic separation of the reaction products was not feasible. Consequently, we decided to investigate activation of the acid **5** in DMSO/CH_2Cl_2, using (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) associated with 1-hydroxybenzotriazole, a method affording high yields in peptide bond synthesis.[23] However, in polar media, the BOP activation method yields several different activated species obtained by successive rearrangements.[24] After several attempts we prepared the porphyrin−spermine adduct **6** in a 54% yield with BOP in DMSO/CH_2Cl_2 in the presence of *N*-methylmorpholine and a 40-fold excess of spermine. The use of a smaller amount of spermine yielded a mixture of **6** and of the product containing two porphyrin moieties for one spermine, a mixture which was difficult to separate. When spermine was introduced in the reaction medium after the BOP addition, a mixture of **6** and a product resulting from the decomposition of the activated ester into an uncharacterized compound was formed. Consequently, it was particularly important to add the BOP reagent after the base, the porphyrin derivative, and the spermine had been mixed, in order to allow the spermine to act as a nucleophile before deactivation of the activated acid by side reactions.

A similar reaction sequence with only 0.5 equiv of spermine led to the porphyrin−spermine conjugate **7** containing two porphyrin moieties. The NMR spectrum of **7** indicated that only the primary amines of spermine reacted under these conditions, yielding the symmetric bis(porphyrin)−spermine conjugate **7**.

Both spermine−porphyrin conjugates **6** and **7** were metalated with manganese(II) chloride to afford the corresponding metalloporphyrins **6**−Mn and **7**−Mn. The synthesis of the hybrid molecule **8**, associating two different porphyrin derivatives, one manganese tetracationic porphyrin (as DNA cleavage reagent), and the corresponding nonmetalated ligand (as intercalating agent) via a spermine linker, was achieved by a coupling reaction between **5**−Mn and **6**. However, **5**−Mn had to be firstly transformed into its hexafluorophosphate salt because the corresponding iodide or chloride salts were not soluble enough in DMSO. This exchange was performed by simply adding KPF_6 to the reaction medium. The formation of compound **8**, with two different porphyrin chromophores, was monitored by HPLC equipped with a diode array detector (RP-Ultrabase column; solvent A, 0.1 M sodium acetate buffer pH 3.2; solvent B, methanol; linear gradient from 100% A to 100% B in 30 min). Both starting materials had retention times and UV−vis spectra (**5**−Mn t_R 3.4 min, λ_{max} 462 nm; **6** t_R 11.5 min, λ_{max} 424 nm) different from those of the coupling product **8** (**8** t_R 14.3 min, two Soret bands at 424 and 462 nm). ES-MS data showed the two expected peaks corresponding to the metalated and the unmetalated porphyrin fragments (Table 2).

All of the new compounds were fully characterized by ^{1}H-NMR, mass spectrometry, and elemental analyses. ES-MS (Table 2) appeared as the most appropriate method for the analysis of such cationic porphyrin compounds as previously discussed.[25]

Nuclease Activity of the Metalloporphyrin−Spermine Conjugates. The nuclease activity of compounds **5**−Mn, **6**−Mn, **7**−Mn, and **8** was investigated on plasmid DNA by agarose gel electrophoresis. The double-stranded supercoiled ΦX174 DNA was incubated with increasing concentrations of **1**−Mn or of the DNA cleaver candidates **5**−Mn, **6**−Mn, **7**−Mn, and **8** and activated with $KHSO_5$ as the oxygen donor.[3a] Both porphyrin derivative and

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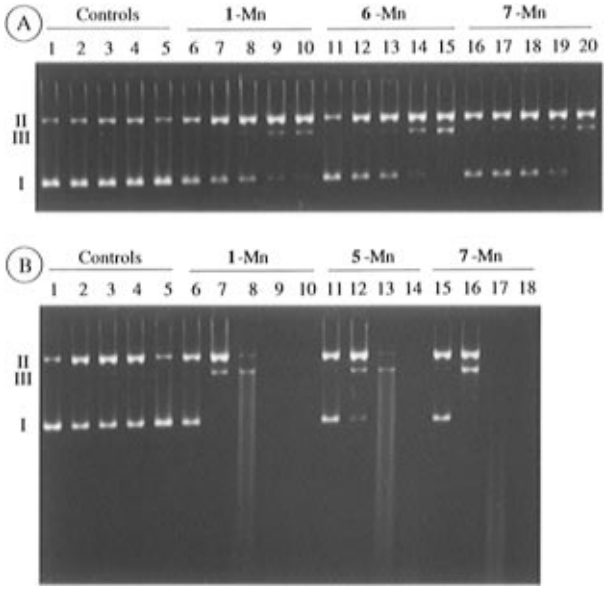

Figure 1. Cleavage of ΦX174 form I DNA by the indicated porphyrin derivatives at 4 μM $KHSO_5$. DNA was electrophoresed on agarose gel and treated as described in the Experimental Section. DNA concentration was 18.7 μM (bp). A and B: (1) DNA control; (2−4) 4 nM **1**−Mn, **6**−Mn (or **5**−Mn), and **7**−Mn controls; (5) 4 μM $KHSO_5$ control. A: (6−10) **1**−Mn 0.5, 0.75, 1, 2, and 4 nM; (11−15) **6**−Mn 0.5, 0.75, 1, 2, and 4 nM; (16−20) **7**−Mn 0.25, 0.38, 0.5, 1, and 2 nM. B: (6−10): **1**−Mn 0.5, 5, 50, 500, and 500 nM; (11−14) **5**−Mn 0.5, 5, 50, and 500 nM; (15−18) **7**−Mn 0.5, 5, 50, and 500 nM. The different forms of plasmid DNA (forms I, II, or III) are indicated in the figure.

oxygen atom donor were required for DNA cleavage: no nuclease activity was observed when the double-stranded supercoiled ΦX174 DNA was incubated solely with either the porphyrin derivative or $KHSO_5$. HEPES buffer, known to inhibit the cleavage reaction by rapid degradation of the oxygen donor,[3a] was added to stop the reaction.

In a comparative study on the different manganese porphyrin complexes, the functionalized tetracationic porphyrin derivative **5**−Mn showed a DNA cleavage activity similar to that of **1**−Mn (Figure 1B, lanes 11−14 compared to lanes 6−10). The introduction of a spermine tether (compound **6**−Mn) did not significantly change the cleavage efficiency in comparison with **1**−Mn (Figure 1A, lanes 11−15 compared to lanes 6−10). Compound **7**−Mn with its two metalated porphyrin moieties was slightly more efficient than **1**−Mn in cleaving ΦX174 DNA (Figure 1A, lanes 16−20 compared to lanes 6−10, or Figure 1B, lanes 15−18 compared to lanes 6−10). The porphyrin conjugate **8**, with one metalated and one nonmetalated porphyrin, was only about half as efficient as **1**−Mn (data not shown), even when the preincubation time of **8** with DNA was increased up to 40 min in order to favor the porphyrin derivative interaction with ΦX174 DNA. At low concentrations (typically 5 nM or lower), **1**−Mn exhibited a catalytic activity and was shown to cleave the double-stranded supercoiled plasmid DNA by means of single-strand breaks (SSBs).[3a] Both compounds **7**−Mn and **8** have a spermine tether able to interact strongly with DNA[10c,d] (in addition **8** bears an unmetalated porphyrin able to intercalate in DNA) and to increase probably the residence time of the catalytical entity at one affinity site; consequently, they might generate two close SSBs (equivalent to a double-strand break). Under experimental conditions usually employed to induce several catalytic cycles (a digestion time of 10 min and 50 μM $KHSO_5$), neither **7**−**Mn** nor **8** induced a form III/form I ratio different from that observed with **1**−Mn. So, such metalloporphyrin−spermine conjugates did not induce targeted double-strand breaks under these experimental conditions.

All of these results indicate that the three tetracationic metalloporphyrin derivatives **5**−Mn, **6**−Mn, and **7**−Mn appeared as DNA cleavers at least as efficient as the parent tetracationic complex **1**−Mn, which was our initial goal. The introduction of a spermine linker (in **6**−Mn and **7**−Mn) did not enhance the cleaving properties of these derivatives in the conditions of the present work on naked DNA, but this polyamine tether was recently shown to improve significantly the cleavage efficiency of double-stranded DNA by metalloporphyrin−linker−oligonucleotide molecules in a triple-helix strategy.[10c,d]

Conclusion

An easy preparation of tetracationic porphyrin−spermine conjugates has been described by using a porphyrin precursor obtained by monoalkylation of the commercially available *meso*-tetrapyridylporphyrin with an ω-bromo aliphatic ester. When activated by potassium monopersulfate, the monofunctionalized tetracationic manganese porphyrin **5**−Mn proved to be an efficient DNA cleaver and allowed spermine conjugates endowed with DNA cleaving properties similar to the ones of the reference chemical nuclease **1**−Mn to be prepared. So we should keep in mind that the synthon **5**−Mn represents a convenient DNA cleaver to be attached to DNA recognition molecules in order to target its cleaving properties on specific sites of DNA. As an illustration, we recently reported that the most efficient metalloporphyrin−oligonucleotide conjugate in DNA cleavage was based on such a tetracationic metalloporphyrin precursor.[26]

Experimental Section

TPyP was synthesized according to a published procedure.[15b,27] Spermine (Sigma), ethyl 5-bromopentanoate, methyl iodide (Acros chemicals), and BOP (Fluka) were used as received. DMSO (Janssen) was dried by distillation and stored over 4 Å molecular sieves.[28] Analytical grade CH_2Cl_2 and 95% ethanol were used. ΦX174 DNA was purchased from Boehringer. Potassium monopersulfate is the triple salt $2KHSO_5$, $KHSO_4$, K_2SO_4. Purifications by column chromatography was performed on silica gel 60 (230−400 mesh). Dowex 1 × 8−200 anion-exchange resin, chloride form (Acros chemicals), was used for ion-exchanges. NMR spectra were recorded in DMSO-d_6 at 250 MHz. ES-MS were recorded on a VG-Trio 2000 instrument after dissolution in H_2O or H_2O/CH_3OH; calculated mass values were obtained without the anions, and data of Table 2 were interpretated according to our previous work on a series of cationic porphyrin derivatives.[25] Elemental analyses were performed at the Laboratory of Coordination Chemistry in Toulouse.

Synthesis of 5-(1-(4-(Ethoxycarbonyl)butyl)pyridinium-4-yl)-10,15,20-tripyridylporphyrin Bromide [3]. 5,10,15,-20-Tetrapyridylporphyrin **2** (1.260 g, 2.04 mmol) was refluxed in a mixture of 5.0 g of ethyl 5-bromopentanoate (23.9 mmol), 100 mL of ethanol, and 300 mL of chloroform for 6 days. After removal of the solvent under reduced pressure, the product was purified by two successive elutions from a short silica gel column (l = 12 cm, CH_2Cl_2/EtOH, 7/3, v/v). The first fraction

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consisted of 310 mg of TPyP, which could be reused. The second red-brown product was recovered and dried under vacuum (yield = 33%, 554 mg). ^{1}H NMR: δ 9.67 (d, 2H, J = 6.4 Hz), 9.24 (d, 2H, J = 5.2 Hz), 9.20 (m, 6H), 9.13 (d, 2H, J = 5.2 Hz), 9.06 (s, 4H), 8.40 (d, 6H, J = 6 Hz, 6H), 5.08 (t, 2H), 4.26 (q, 2H), 2.68 (t, 2H), 2.41 (m, 2H), 1.97 (m, 2H), 1.37 (t, 3H), −2.93 (s, 2H). Anal. Calcd for $C_{47}H_{39}N_8O_2Br\cdot 2H_2O$: C, 65.35; H, 5.02; N, 12.97. Found: C, 65.37; H, 4.89; N, 12.91. Visible (ethanol): λ_{max} 416 nm, $\epsilon = 2.2 \times 10^5$ M^{-1} cm^{-1}.

Synthesis of 5-(1-(4-(Ethoxycarbonyl)butyl)pyridinium-4-yl)-10,15,20-tris(1-methylpyridinium-4-yl)porphyrin Bromide, Triiodide (4). 3 (500 mg, 0.604 mmol) was dissolved in 60 mL of DMSO, and 650 μL (10.4 mmol) of methyl iodide was added. The mixture was heated to 42 °C for 2 h, and a new portion of methyl iodide (650 μL) was added. Stirring and heating was continued for 2 h, and the solvent was evaporated under vacuum. The product was dried under reduced pressure to afford 740 mg (yield = 100%) of **4**. This product was pure enough to be used in the next step. An analytical sample was prepared by dissolving 10 mg of the solid in 1 mL of water, filtration, and precipitation of **4** with 5 mL of 2-propanol and 1 mL of diethyl ether and drying under reduced pressure. ^{1}H NMR: δ 9.70 (d, 2H, J = 6.5 Hz), 9.61 (d, 6H, J = 6.5 Hz); 9.39 (d, 2H, J = 4.7 Hz), 9.35 (s large, 6H), 9.13 (m, 8H); 5.10 (t, 2H), 4.86 (s, 9H), 4.27 (q, 2H), 2.68 (t, 2H), 2.44 (m, 2H), 2.00 (m, 2H), 1.38 (t, 3H), −2.98 (s large, 2H). Anal. Calcd for $C_{50}H_{48}N_8O_2BrI_3\cdot 3H_2O$: C, 45.93; H, 4.16; N, 8.57. Found: C, 46.24; H, 4.61; N, 8.02. Visible (H_2O): λ_{max} = 422 nm, $\epsilon = 1.7 \times 10^5$ M^{-1} cm^{-1}.

Synthesis of 5-(1-(4-Carboxybutyl)pyridinium-4-yl)-10,15,20-tris(1-methylpyridinium-4-yl)porphyrin Bromide, Triiodide (5). 4 (900 mg, 0.72 mmol) was dissolved in 130 mL of 1 M HCl and refluxed for 1 h. The mixture was allowed to cool and filtered on a sintered glass filter no. 4. The solvent was evaporated, and water (50 mL) was added and evaporated three times to ensure the complete elimination of HCl. After the mixture was dried under vacuum, 850 mg of the acid **5** was obtained (yield = 91%). ^{1}H NMR: δ 9.71 (d, 2H, J = 6.1 Hz), 9.62 (d, 6H, J = 5.7 Hz), 9.39 (d, 2H, J = 4.9 Hz), 9.32 (s large, 6H), 9.13 (m, 8H), 5.10 (t, 2H, J = 6.1 Hz), 4.85 (s, 9H), 2.70 (t, 2H), 2.46 (m, 2H), 1.96 (m, 2H), −2.98 (s large, 2H). Anal. Calcd for $C_{48}H_{44}N_8O_2BrI_3\cdot 4H_2O$: C, 44.43; H, 4.04; N, 8.64. Found: C, 44.56; H, 3.77; N, 8.62. Visible (H_2O): λ_{max} = 422 nm, $\epsilon = 1.7 \times 10^5$ M^{-1} cm^{-1}.

Synthesis of Manganese(III) 5-(1-(4-Carboxybutyl)-pyridinium-4-yl)-10,15,20-tris(1-methylpyridinium-4-yl)-porphyrin Bromide, Chloride, Triiodide (5−Mn). 5 (47 mg, 36.1 μmol) was dissolved in 3 mL of water. Manganese-(II) chloride tetrahydrate (16 mg, 81 μmol) was added, and the solution was refluxed for 80 min. The metalation reaction was monitored by visible spectroscopy (the Soret band shifted from 420 to 464 nm). After complete metalation, the reaction mixture was poured into 60 mL of a 50/50 mixture of 2-propanol and diethyl ether. The precipitate was recovered by centrifugation, washed with diethyl ether, and dried. Yield: 40 mg (74.9%). Anal. Calcd for $C_{48}H_{42}N_8O_2BrClI_3Mn\cdot 4H_2O$: C, 41.60; H, 3.64; N, 8.08. Found: C, 41.43; H, 3.53; N, 8.12. Visible (H_2O): λ_{max} = 464 nm, $\epsilon = 1.15 \times 10^5$ M^{-1} cm^{-1}.

Synthesis of Porphyrin−Spermine Conjugate (6) (Tetrachloride, Trihydrochloride Form). 5 (100 mg, 77.1 μmol) was placed into 20 mL of freshly distilled and dried DMSO. *N*-Methylmorpholine (400 μL, 3.64 mmol), spermine (600 mg, 2.97 mmol), and CH_2Cl_2 (6 mL) were added, and the solution was stirred until all of the solids were dissolved. (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) (160 mg, 362 μmol) was added, and the reaction mixture was stirred for 30 min. Water (2 mL) was added, followed by 60 mL of 2-propanol and 60 mL of diethyl ether. The precipitate was recovered by centrifugation, washed with 10 mL of 2-propanol, and dried under reduced pressure. This solid **6** (83 mg, 50.4 μmol, yield = 65%) (mixed anions form) was pure enough to be used in the next step (synthesis of **8**). Anal. Calcd for $C_{58}H_{68}N_{12}OBrI_3\cdot HPF_6\cdot 5H_2O$: C, 42.33; H, 4.84; N, 10.21. Found: C, 42.63; H, 4.66; N, 9.85. Visible (H_2O): λ_{max} = 422 nm, $\epsilon = 2.0 \times 10^5$ M^{-1} cm^{-1}. This product could be further purified by dissolving it in 3 mL of water and precipitation by the subsequent addition of 60 mL of 2-propanol and 10 mL of diethyl ether. Finally, the anions can be exchanged for chloride anions by passage of the aqueous solution (2 mL) on 5 mL of DOWEX-1 × 8−200 ion exchange resin (chloride form). The solvent was evaporated and the solid dried under vacuum to afford 55 mg (54%) of a slightly hygroscopic solid. ^{1}H NMR: δ 9.73 (d, 2H, J = 6.1 Hz), 9.61 (d, 6H, J = 6.1 Hz), 9.40 (d, 2H, J = 4.2 Hz), 9.33 (m large, 6H, large), 9.14 (m, 8H), 8.26 (t, 1H, J = 4.9 Hz), 5.14 (t, 2H, J = 7.5 Hz), 4.86 (s, 9H), 3.28 (m, 2H), 3.05 (m, 10H), 2.49 (t, 2H, J = 7.4 Hz), 2.40 (m, 2H), 2.07 (m, 2H), 1.98 (s, large, 4H), 1.83 (s large, 4H), −2.98 (s large, 2H). Anal. Calcd for $C_{58}H_{68}N_{12}OCl_4\cdot 3HCl\cdot 8H_2O$: C, 51.81; H, 6.52; N, 12.50. Found: C, 52.36; H, 6.49; N, 12.13. Visible (H_2O): λ_{max} = 422 nm, $\epsilon = 2.0 \times 10^5$ M^{-1} cm^{-1}.

Synthesis of Manganese(III) Porphyrin−Spermine Conjugate (6−Mn) (Mixed Anions Form). 6 (mixed anions form, 20 mg, 11.2 μmol) and 5.0 mg of $MnCl_2\cdot 4H_2O$ (25.3 μmol) were dissolved in 1 mL of water and refluxed for 1 h. The metalation reaction was monitored by visible spectroscopy (shift of the Soret band from 422 to 462 nm). The metalated porphyrin was precipitated by the addition of 20 mL of a 50/50 mixture of 2-propanol and diethyl ether; 13 mg (yield = 64%) of **6**−Mn, mixed anions form, was obtained. Anal. Calcd for $C_{58}H_{67}N_{12}OMnBrClI_3\cdot HPF_6.9H_2O$: C, 38.54; H, 4.80; N, 9.30. Found: C, 38.74; H, 4.86; N, 8.96. Visible (H_2O): λ_{max} = 462 nm, $\epsilon = 1.2 \times 10^5$ M^{-1} cm^{-1}. Exchange of the anions on a Dowex 1 × 8−200 ion exchange resin, chloride form, yielded a very hygroscopic solid unsuitable for elemental analysis.

Synthesis of Bis(porphyrin)−spermine conjugate 7 (Dibromide, Hexaiodide, Dihexafluorophosphate Form). Crude **5** (mixed anions form, 30 mg, 23.1 μmol) and 2.30 mg of spermine (11.3 μmol) were dissolved in 6 mL of DMSO. *N*-Methylmorpholine (100 μL, 910 μmol) and 0.6 mL of CH_2Cl_2 were added, followed by the addition of 60 mg (13.6 μmol) of BOP. The reaction medium was stirred for 20 min, and 55 mL of 2-propanol was added. The precipitate was recovered by centrifugation, washed with 5 mL of 2-propanol, dissolved in 5 mL of H_2O/EtOH (50/50), and filtered through a no. 4 sintered glass filter. The filtrate was recovered and the solvent evaporated. The obtained solid was recrystallized from a minimum of hot water. Yield = 61% (22 mg). ^{1}H NMR: δ 9.64 (d, 4H, J = 6.1 Hz), 9.54 (d, 12H, J = 6.1 Hz), 9.36 (d, 4H, J = 4.2 Hz), 9.28 (m, 12H), 9.09 (m, 16H), 8.28 (t, 2H, J = 4.9 Hz), 5.08 (t, 4H, J = 7.5 Hz), 4.83 (s, 18H), 3.31 (m, 4H), 3.06 (t large, 8H), 2.48 (t, 4H, J = 7.4 Hz), 2.38 (m, 4H), 1.96 (m, 8H), 1.82 (s large, 4H), −2.98 (s large, 2H). Anal. Calcd for $C_{106}H_{110}N_{20}O_2Br_2I_6\cdot 2HPF_6\cdot 12H_2O$: C, 40.73; H, 4.39; N, 8.96. Found: C, 40.77; H, 4.52; N, 8.70. Visible (H_2O): λ_{max} = 422 nm, $\epsilon = 3.6 \times 10^5$ M^{-1} cm^{-1}.

Synthesis of Bis[manganese(III) porphyrin]−Spermine Conjugate 7−Mn (Dibromide, Dichloride, Hexaiodide, Dihexafluorophosphate Form). 7 (12 mg, 3.84 μmol) and 4.5 mg (22.7 μmol) of $MnCl_2\cdot 4H_2O$ were refluxed in 3 mL of water for 8 h. The metalation reaction was monitored by visible spectroscopy (shift of the Soret band from 422 to 462 nm). The reaction mixture was filtered, and 40 mL of 2-propanol/diethyl ether (50/50) was added to the filtrate. The precipitate which appeared at this moment was recovered by centrifugation, washed with 12 mL of 2-propanol, and redissolved in approximately 1 mL of water. The metalated porphyrin was precipitated by the subsequent addition of 4 mL of 2-propanol and 5 mL of diethyl ether. **8**−Mn yield = 62% (8 mg, 2.40 μmol). Anal. Calcd for $C_{106}H_{106}N_{20}O_2Mn_2Br_2Cl_2I_6\cdot 2HPF_6\cdot 14H_2O$: C, 38.14; H, 4.11; N, 8.39. Found: C, 38.16; H, 3.95; N, 8.24. Visible (H_2O): λ_{max} = 462 nm, $\epsilon = 2.4 \times 10^5$ M^{-1} cm^{-1}.

Synthesis of Monomanganese(III) Complex of Bis-(porphyrin)−Spermine Conjugate 8 (Nonachloride, Dihydrochloride Form). Crude **6** (bromide/triiodide form, 5.7 mg, 3.46 μmol), 4.1 mg (2.96 μmol) of **5**−Mn, 10 mg (54 μmol) of KPF_6, and 50 μL (455 μmol) of *N*-methylmorpholine were dissolved in 0.8 mL of DMSO, followed by the addition of 0.2 mL of CH_2Cl_2 and 25 mg (56.5 μmol) of BOP, and the solution was stirred for 1 h at room temperature. Water (2 mL) and 2-propanol (10 mL) were added, and the precipitate was

recovered by centrifugation, washed once with 2-propanol, dissolved in 1 mL of a 60/40 water/acetone mixture, and eluted through a DOWEX 1 × 8−200 ion exchange resin (Cl^- form). The solvent was evaporated and the product dried under vacuum to yield 4.9 mg of **8**−Mn. Visible (H_2O): $\lambda_{max} = 422$ nm, $\epsilon = 1.7 \times 10^5\ M^{-1}\ cm^{-1}$; $\lambda_{max} = 462$ nm, $\epsilon = 8.8 \times 10^4\ M^{-1}\ cm^{-1}$. Anal. Calcd $C_{106}H_{108}N_{20}O_2MnCl_9 \cdot 2HCl \cdot 5DMSO \cdot 24H_2O$: C, 47.04; H, 6.33; N, 9.46. Found: C, 47.30; H, 6.33; N, 9.14.

DNA cleavage experiments were performed according to ref 3a. Briefly, the reaction involved 12.5 μg/mL (3.5 nM) of supercoiled ΦX174 DNA, 50 mM phosphate buffer pH 8, 100 mM NaCl, porphyrin derivatives (0.5 to 500 nM), and $KHSO_5$ (4 μM final concentrations) in a total volume of 10 μL. Preincubation of DNA and porphyrin derivatives was for 10 or 40 min at 20 °C. Addition of $KHSO_5$ initiated the cleavage reaction. Unless otherwise stated, the digestion time was 1 min at 20 °C. One microliter of 1 M HEPES, pH 8, was added to stop the reaction. Bromophenol blue was added to the reaction mixtures, and electrophoresis in 0.8% agarose gel containing 1 μg/mL of ethidium bromide was performed at constant current (25 mA for 15 h) in 89 mM Tris-borate buffer and 2.5 mM EDTA. Bands were located by UV light (254 nm) and photographed.

Acknowledgment. This work was supported by ANRS (French Agency for Research on AIDS), ARC (Association pour la Recherche contre le Cancer, Villejuif, France), and CNRS. One of us (A.J.) is indebted for a postdoctoral fellowship from the European Union (Human Capital and Mobility program).

Supporting Information Available: Figure 2 with ^1H NMR (in DMSO-d_6) chemical shift assignments for compounds **3**−**7** (1 page). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9620831