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Preparation and characterization by electrospray mass spectrometry of cationic metalloporphyrin DNA cleavers†

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Summary — Cationic metalloporphyrin derivatives endowed with nuclease activity have been prepared and characterized by electrospray mass spectrometry. These metalloporphyrin precursors have various functionalized tethers and are excellent DNA cleavers to be linked to an oligonucleotide or a minor groove binder like Hoechst 33258, a bis-benzimidazole DNA dye.

cationic metalloporphyrin / functionalized tether / Hoechst 33258 conjugate / electrospray mass spectrometry

Résumé — Préparation et caractérisation par spectrométrie de masse en électrospray d'une série de métalloporphyrines cationiques, agents de coupure de l'ADN. *Une série de métalloporphyrines cationiques à activité nucléase a été préparée et caractérisée par spectrométrie de masse en électrospray. Ces précurseurs porphyriniques fonctionnalisés sont d'excellents agents de coupure de l'ADN qui peuvent être liés à un oligonucléotide ou, comme cela est décrit, à Hoechst 33258, un colorant capable de reconnaître le petit sillon de l'ADN.*

métalloporphyrine cationique / bras fonctionnalisé / Hoechst 33258 conjugué / spectrométrie de masse par électrospray

Introduction

Oxidative DNA cleavage by transition metal complexes (iron-EDTA, copper-phenanthroline, nickel-cyclam, ruthenium-bipyridine or metalloporphyrin derivatives, see ref [1–5] for recent leading references and ref [6, 7] for two reviews on DNA cleavage) is a growing domain in biological chemistry because of the possible development of these DNA cleavers in two different application fields: (i) preparation of artificial nucleases for the site-specific cleavage of genomic DNA; and (ii) design of new potential antitumoral and antiviral agents. Cationic manganese porphyrins bearing *N*-methylpyridiniumyl substituents at the *meso* positions of the tetrapyrrolic macrocycle have a high affinity for nucleic acids and can cleave single- or double-stranded DNA when activated in vitro by potassium monopersulfate, a water-soluble inorganic peroxide [5]. Such artificial nucleases are expected to be activated inside cells by a reducing agent and molecular oxygen as previously observed for bleomycin, an antitumoral agent using iron, dioxygen and two electrons as cofactors [8]. In the present article we report the preparation of unsymmetrical cationic metalloporphyrins bearing convenient tethers for covalent linkage to intercalators [9], oligonucleotides [5, 10] or a minor groove binder like Hoechst 33258 (HOE 33258; 4-[5-(4-methyl-1-piperazinyl)[2,5′-bi-1*H*-benzimidazol]-2′-yl]phenol trishydrochloride), a bis-benzimidazole derivative used as DNA dye [11]. Since cationic metalloporphyrins cannot be characterized by classical desorption methods used to obtain mass spectrometry data (electronic impact (EI), desorption chemical ionization (DCI) or fast atom bombardment (FAB)), we also report the characterization of the positively charged complexes by electrospray mass spectrometry (ES-MS).

Results and discussion

Synthesis of porphyrin precursor **1**
(see fig 1 for structure)

The synthesis of a cationic porphyrin with three *N*-methylpyridiniumyl substituents at the *meso* positions of the macrocycle and a protected phenoxy group has been described previously, but the purification of the desired isomer of this unsymmetrical porphyrin derivative was rather tedious [12]. We report here an improved preparation of **1** from a mixture of 4-hydroxybenzaldehyde, 4-pyridinecarboxaldehyde and pyrrole refluxed in propanoic acid containing a small

† Dedicated to Prof Raymond Weiss.

* Correspondence and reprints

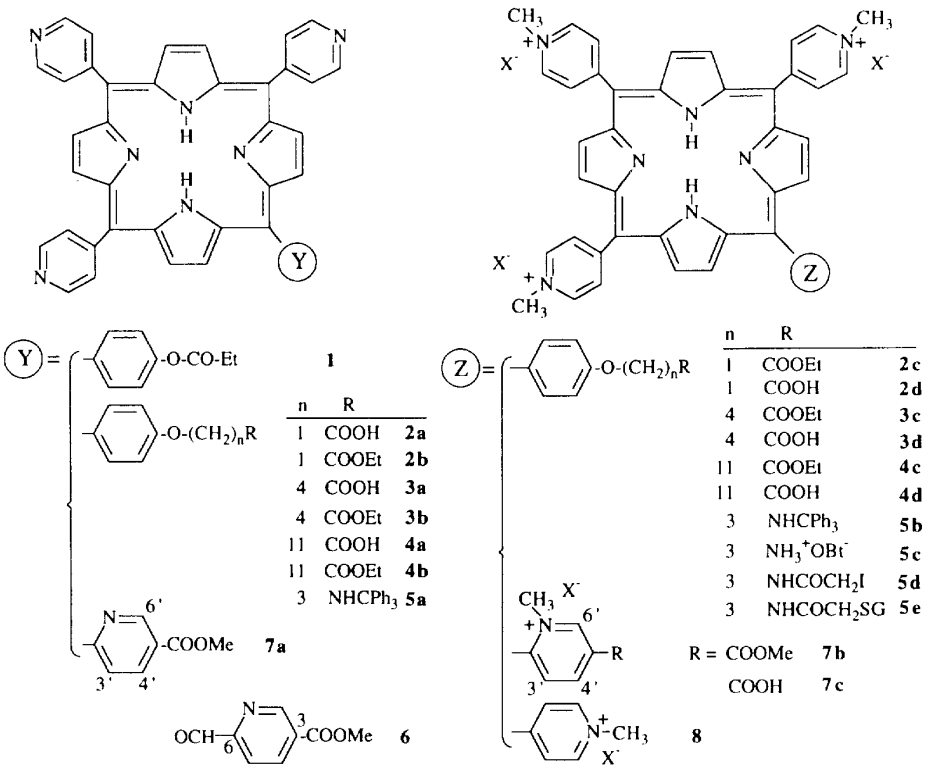

Fig 1. Structures of the porphyrin ligands. The corresponding metalloporphyrins are indicated by the metal abbreviation as suffix, eg, **4d-Mn** stands for the manganese derivative of porphyrin **4d**. For the nature of X^-, see table I.

amount of acetic anhydride. The reaction mixture containing all the six porphyrin isomers (tetrapyridyl, tripyridyl, *cis* and *trans* dipyridyl, monopyridyl and nopyridyl isomers) and tar was neutralized with a $KHCO_3$ solution, filtered and dried under reduced pressure. The resulting black solid was dissolved in dichloromethane, and hexane was added (the final mixture is CH_2Cl_2/hexane, 55:45, v/v). Only tar precipitated. The supernatant was recovered and dried under reduced pressure. The remaining black powder was then dissolved in a mixture of ethanol and dichloromethane and precipitated by hexane addition (final EtOH/CH_2Cl_2/hexane mixture = 47:6:47, v/v/v). The pellet contained only the six porphyrin isomers as a purple powder (all chlorins and residual tars were eliminated). The porphyrin mixture was dissolved in dichloromethane and purified by silica-gel chromatography. The desired isomer **1** with three pyridyl and one propanoyloxyphenyl motifs was obtained with a 2–3% yield.

Synthesis of tris(methylpyridiniumyl)metalloporphyrins having a tether functionalized with an acid function

The precursor **1** was used as a starting material for the preparation of different unsymmetrical cationic metalloporphyrins with a tether bearing a carboxylic acid function or an iodoacetyl function, in order to be conjugated with an oligonucleotide modified with a primary amine or a thiol linker, respectively.

Porphyrins **2a** and **4a** were obtained by alkylation of **1** with the appropriate halide derivative, respectively, iodoacetic acid and 12-bromododecanoic acid, following a procedure that was previously described for the ester **3b** [12]. Protection of the terminal carboxylic acid function, which is required before alkylation of the three pyridine moieties by methyl iodide, was performed by refluxing a solution of **2a** or **4a** in acidic ethanol and gave ethyl esters **2b** or **4b** in 80% yield. The next steps of the synthesis (ie, methylation, deprotection of the acid function and metallation) were performed as previously reported [12] and led to **2c**, **2d** and **2d-Mn** or to **4c**, **4d** and **4d-Mn**, respectively.

Synthesis of tris(methylpyridiniumyl) metalloporphyrins functionalized with an amine group and related acetylated derivatives

Compounds **5a**, **5b** and **5b-Mn** were synthesized as previously described [12]. These tricationic porphyrin derivatives are functionalized with a tritylated primary amine residue. Amine deprotection was performed with 1-hydroxybenzotriazole (HOBt) in trifluoroethanol since HOBt is acidic enough to cleave a trityl group [13]. This quantitative reaction led to the benzotriazolate salt of the corresponding porphyrin derivatives (**5c** and **5c-Mn**, respectively) which can be engaged with an anhydride without further purification [13]. For example, **5c** (or **5c-Mn**) reacted with iodoacetic anhydride [14] to give the iodoacetylated tricationic derivative **5d** (or **5d-Mn**) in 95% yield. Iodoacetyl-containing

molecules are powerful agents for protein modification since they can react under mild conditions with cysteine residues [15]. Reactivity of **5d** was checked by reaction with glutathione (Glu-Cys-Gly), giving the tricationic porphyrin-glutathione compound **5e** which was characterized by ES-MS.

Synthesis of a functionalized tetrakis(methylpyridiniumyl)metalloporphyrin

Tetrakis(methylpyridiniumyl) porphyrins, with one more positive charge than the corresponding tris(methylpyridiniumyl) derivatives, have a higher affinity for DNA [16]. In preliminary research on DNA cleavage by metalloporphyrin-oligonucleotide conjugates, we always used the **3d-Mn** precursor which has only three positive charges on the macrocycle ligand, not four charges like Mn-TMPyP (**8-Mn**), a minor groove binder that can cleave DNA by hydroxylation of deoxyribose C–H bonds [5a,b]. Since this symmetrical metalloporphyrin does not exhibit peripheral reactive function, it cannot be conjugated to any vectors. Consequently, we decided to synthesize the unsymmetrical precursor **7c** which has four positive charges to provide a high DNA affinity and a tether with a terminal acid function for conjugation. The synthesis of the unsymmetrical porphyrin **7a** with three pyridyl and one nicotinyl *meso*-substituent was carried out in propanoic acid in the presence of acetic anhydride according to a slightly modified version of the classical Adler-Longo method [12]. The 6-formyl derivative of nicotinic acid was prepared by oxidation of methyl 6-methylnicotinate to methyl 6-formylnicotinate **6** with iodine, *t*-butyl iodide and trifluoroacetic acid in DMSO [17] (see also ref [18] for another preparation of **6**). The ester-protected porphyrin **7a** was obtained after precipitation of tars and separation and purification of porphyrin derivatives by silica-gel chromatography. The final yield of **7a** was 1%. As methylation of **7a** at room temperature with an excess of methyl iodide led only to alkylation of the three 4-pyridyl substituents, then the methylation step was performed with methyl *p*-toluenesulfonate in refluxing DMF in order to achieve the complex methylation of the four pyridyl sites (porphyrin **7b**). Deprotection of the acid function of **7b** was performed in 6 M HCl at 75 °C over 6 h. In order to displace the equilibrium, methanol resulting from ester hydrolysis was eliminated during the reaction by a gentle nitrogen stream. Compound **7c** was obtained without purification after solvent evaporation. We are currently using the corresponding manganese derivative of this functionalized tetracationic porphyrin to prepare modified oligonucleotides. Such cationic metalloporphyrins can also be linked to a DNA minor-groove binder (see below).

Electrospray mass spectrometry data on cationic porphyrin derivatives **2–8**

Hydrophobic porphyrin derivatives are easily characterized by elemental analyses and usual mass spectrometry (DCI or FAB), but several factors limit the full characterization of non-volatile cationic porphyrin derivatives: (i) due to their poor combustion properties, elemental analyses are often out of the admitted range for errors; (ii) the manganese(III) and iron(III) derivatives, the most efficient cationic metalloporphyrins in DNA cleavage, are paramagnetic, making a complete interpretation of NMR spectra impossible; and (iii) mass spectrometry data are not available for these cationic molecules. FAB-MS and ^{252}Cf-plasma desorption MS data are only accessible for non-charged metalloporphyrins [19]. Consequently, the last stage of complete characterization of porphyrin precursors used in synthesis of hybrid 'cationic metalloporphyrin-vector' molecules is the step before alkylation of pyridine residues and metallation of the cationic macrocycle. Here we report data on the electrospray ionization method which allows a full characterization of these cationic metallated porphyrin compounds. Electrospray mass spectrometry (ES-MS) recently emerged as a powerful technique for the measurement of molecular masses of non-volatile compounds that would be fragmented under classical ionization methods [20]. For example, ES-MS has been successfully applied to analysis of protein complexes [21a], fullerene derivatives [21b] and catenanes based on copper compounds [21c].

ES-MS data of different cationic porphyrin derivatives have been collected. The compounds listed in table I include free porphyrin ligands and some of their iron, manganese or nickel complexes. Interpretations of the main detected peaks are presented below.

Case of free porphyrin ligands

The detected molecular peaks (see table I for masses of calculated and observed peaks) are mainly cationic species corresponding to the exact number of positive charges of the porphyrin derivatives: peaks corresponding to $m/3$ were detected for the tricationic compounds **2c**, **2d**, **3c**, **3d**, **4c**, **4d**, **5b**, **5c**, **5d** and **5e**, to $m/4$ for the tetracationic derivatives **7b**, **7c** and **8**. Furthermore, the overall charge of the molecule may be lowered by one unit by one of the following: (i) a one-electron reduction giving molecular peaks at $m^-/2$ in the case of tricationic compounds **2c**, **3c**, **4c**, **5b**, **5c** and **5d**; (ii) the loss of one proton giving a peak at $m - H^+/2$ or $m - H^+/3$ in the case of tricationic or tetracationic derivatives, respectively, when a free carboxylic group is present (see **2d**, **3d**, **4d** and **7c**); or (iii) the loss of one CH_3^+ giving a peak at $m - CH_3^+/2$ (**2c**) or $m - CH_3^+/3$ (**8**). A one-electron reduction of non-metallated porphyrins is easily accessible in solution (see ref [23a] for reduction potentials of cationic methylpyridinium-porphyrins, ref [23b] for cyanoporphyrins and ref [23c] for one- or two-electron reduction potentials of iron porphyrin complexes). Proton loss can also be observed in the case of the tetracationic porphyrin **8** (main observed peak at 226.0 corresponding to $m - H^+/3$, see table I); such deprotonation on a porphyrin without carboxylic acid function is supposed to be due to proton release from one pyrrolic N–H inside of the macrocycle. This phenomenon was also observed for **7b** when the carboxylic function of the linker is blocked as an ester. In some specific cases, fragmentations could be also observed: the loss of trityl protect-

Table I. Electrospray mass spectrometry data on cationic porphyrin derivatives **2–8**.

Compd	*M*	X^-	*Linker (n, R)*	*Formula*	*MW*[g] m	*molecular peak(s)*[h] m/z	*calc*	*obs*	*Other peak(s)*[h] m/z	*calc*	*obs*	Ref prep
2c	H_2	I^-	1, COOEt	$C_{48}H_{42}N_7O_3$	764.90	*m/3*	255.0	255.1	*m-CH*$_3^+$*/2*	374.9	374.9	*i*
						$m^-/2$	382.4	382.4				
2d	H_2	I^-	1, COOH	$C_{46}H_{38}N_7O_3$	736.85	*m/3*	245.6	245.7	*m-H*$^+$*/2*	367.9	368.1	*i*
2d-Mn	Mn^{III},Cl^-	I^-	1, COOH	$C_{46}H_{36}N_7O_3Mn$	789.77	$m^-/3$	263.3	263.4	*m+Cl*$^-$*/3*	275.1	275.1	*i*
3c	H_2	I^-	4, COOEt	$C_{51}H_{48}N_7O_3$	806.99	*m/3*	269.0	269.1	*m-CH*$_3^+$*/2*	396.0	395.9	*12*
						$m^-/2$	403.5	403.4				
3c-Ni[a]	Ni^{II}	I^-	4, COOEt	$C_{51}H_{46}N_7O_3Ni$	862.97	*m/3*	287.7	287.7				*12*
3d	H_2	I^-	4, COOH	$C_{49}H_{44}N_7O_3$	778.94	*m/3*	259.7	259.9	*m-H*$^+$*/2*	389.0	389.1	*12*
3d-Mn	Mn^{III},Cl^-	I^-	4, COOH	$C_{49}H_{42}N_7O_3Mn$	831.86	$m^-/3$	277.3	277.4	*PorPhO/3*	243.6	243.5	*12*
									m+Cl$^-$*/3*	289.1	289.2	
									m^-*-H*$^+$*/2*	415.4	415.1	
3d-Fe[b]	Fe^{III}	I^-	4, COOH	$C_{49}H_{42}N_7O_3Fe$	832.77	$m^-/3$	277.6	277.8	*PorPhOH/3*	244.2	244.4	*12*
									m^-*-H*$^+$*/2*	415.9	415.5	
									m+SO$_4^{2-}$*/2*	464.4	464.5	
4c	H_2	I^-	11, COOEt	$C_{58}H_{62}N_7O_3$	905.17	*m/3*	301.7	301.7				*i*
						$m^-/2$	452.6	452.4				
4d	H_2	I^-	11, COOH	$C_{56}H_{58}N_7O_3$	877.12	*m/3*	292.4	292.3	*m-H*$^+$*/2*	438.1	438.3	*i*
4d-Mn	Mn^{III}, Cl^-	I^-	11, COOH	$C_{56}H_{56}N_7O_3Mn$	930.04	*m/4*	232.5	232.5	*PorPhO/3*	243.6	243.6	*i*
						$m^-/3$	310.0	309.8				
5b[c]	H_2	I^-	3, $NHCPh_3$	$C_{66}H_{57}N_8O$	978.23	*m/3*	326.1	325.9	*PorPhOH/3*	226.3	226.3	*12*
						$m^-/2$	489.1	489.1	*m-trit/3*	245.3	245.4	
									PorPhO/2	338.9	338.9	
									m^-*-trit/2*	368.0	367.9	
5b-Mn[c]	Mn^{III}, Cl^-	I^-	3, $NHCPh_3$	$C_{66}H_{55}N_8OMn$	1031.15	$m^-/3$	343.7	343.7	m^-*-trit/3*	262.9	263.0	*12*
									m^-*-trit/2*	393.9	394.2	
									m^{2-}*-trit+I*$^-$*/2*	457.9	457.7	
									m^- *+I*$^-$*/2*	579.0	578.8	
5c[d]	H_2	I^-	3, NH_2	$C_{47}H_{43}N_8O$	735.91	*m/3*	245.3	245.2	*PorPhOH/3*	226.3	226.2	*12*
						$m^-/2$	368.0	367.9				
5c-Mn[d]	Mn^{III},Cl^-	I^-	3, NH_2	$C_{47}H_{41}N_8OMn$	788.83	$m^-/3$	262.9	262.9	m^- *+CH*$_2$*/3*	267.6	267.6	*i*
									m^- *+I*$^-$*/2*	457.9	457.7	
5d	H_2	I^-	3, $NHCOCH_2I$	$C_{49}H_{44}N_8O_2I$	903.84	*m/3*	301.3	301.5	*PorPhOH/3*	226.3	226.2	*i*
						$m^-/2$	451.9	452.0	*PorPhO/2*	338.9	339.0	
5d-Mn	Mn^{III}, Cl^-	I^-	3, $NHCOCH_2I$	$C_{49}H_{44}N_8O_2IMn$	956.76	$m^-/3$	318.9	319.0	*PorPhOH/3*	243.9	243.8	*i*
									m^-*-COCH*$_2$*I/3*	262.9	262.9	
									PorPhO/2	365.4	365.2	
5e[e]	H_2	I^-	3, $NHCOCH_2SG$	$C_{59}H_{60}N_{11}O_8S$	1083.27	*m/3*	361.1	361.1	*PorPhOH/3*	226.3	226.3	*i*
7b	H_2	OTs^-[f]	COOMe	$C_{46}H_{40}N_8O_2$	736.88	*m/4*	184.2	184.1	*m-H*$^+$*/3*	245.3	245.2	*i*
									m+OTs$^-$*/3*	302.7	302.5	
									m+2OTs$^-$*/2*	539.6	539.4	
7c	H_2	OTs^-[f]	COOH	$C_{45}H_{38}N_8O_2$	722.85	*m/4*	180.7	181.0	*m-H*$^+$*/3*	240.6	240.7	*i*
8	H_2	OAc^-	–	$C_{44}H_{38}N_8$	678.84	*m/4*	169.7	169.8	*m-CH*$_3^+$*/3*	221.3	221.1	*22*
									m-H$^+$*/3*	225.9	226.0	
									m-H$^+$*-CH*$_3^+$*/2*	331.4	331.4	
									m-2H$^+$*/2*	338.4	338.4	
8-Mn	Mn^{III},OAc^-	OAc^-	–	$C_{44}H_{36}N_8Mn$	731.76	*m/5*	146.4	146.4	*m-CH*$_3^+$*/4*	179.2	179.2	*22*
						$m^-/4$	182.9	182.9	*m-2CH*$_3^+$*/3*	233.9	233.9	
						$m^{--}/3$	243.9	243.9	m^-*-CH*$_3^+$*/3*	238.9	238.9	

[a] Masses were calculated using the mass of the main isotope of nickel (m = 58). [b] The extra counterion is a mixture of sulfate and chloride. [c] Loss of the trityl protecting group (trit) was accompanied by protonation of the remaining R-NH^- fragment. [d] HOBt salt. [e] GS = Glutathione. [f] OTs^- stands for the *p*-toluenesulfonate anion. [g] m is calculated without the counterions and, in the case of metallated derivatives, without the axial ligands and the extra counterion if present. [h] The 100% peak is underlined; m^- corresponds to the one-electron reduction product; PorPhO and PorPhOH represent unprotonated and protonated fragments when the linker is cleaved at the phenolic position of the functionalized porphyrin. [i] This work.

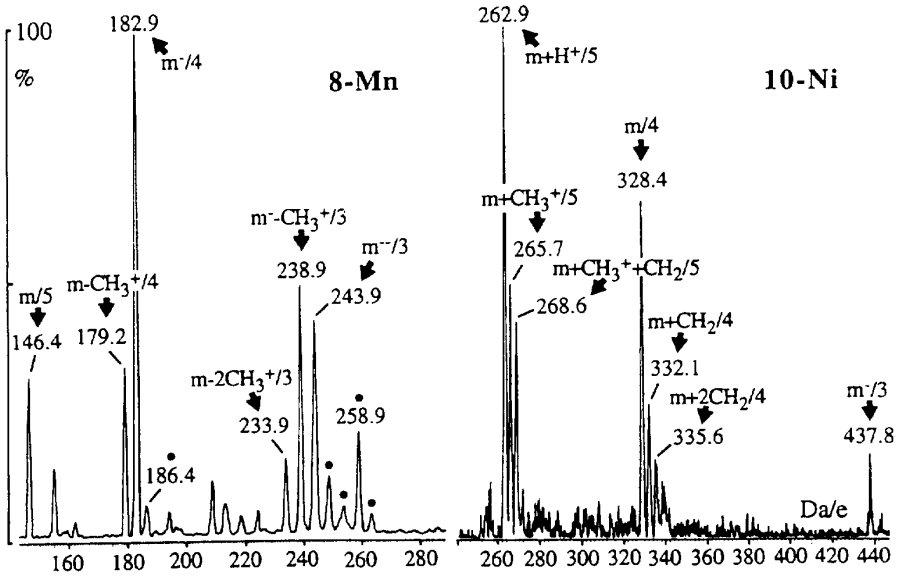

Fig 2. Electrospray mass spectra of compounds **8-Mn** and **10-Ni** (dotted peaks probably correspond to permethylation reactions during analysis).

ing group (accompanied by the protonation of the basic terminal R-NH^- fragment) was detected for both the tricationic (m - trit/3) and the dicationic (one-electron reduced form, m^- - trit/2) compound **5b**; the trityl group itself was observed at 243.3, calculated 243.3. As other examples, **5b**, **5c**, **5d** and the glutathione derivative **5e** gave fragments (PorPhOH/3 or PorPhO/2) corresponding to the loss of the pendant substituent at the phenolic position on the *meso* phenyl group of the porphyrin derivative. Additionally, in two cases we detected peaks corresponding to the loss of one methyl from the methylpyridiniumyl motif accompanied by a reduction of one unit of the charge of the fragment (fragments at $m - CH_3^+/2$ and $m - CH_3^+/3$ for **2c** and **8**, respectively, table I).

Case of cationic metalloporphyrins

Cationic metalloporphyrin complexes like **8-Mn** have been isolated in the solid state with two water molecules as axial ligands and an extra counterion to balance the $[Mn^{III}(H_2O)_2]^+$ motif (see ref [24] for the recent X-ray structure of **8-Mn**, the manganese^{III} derivative of H_2TMPyP). In fact, the molecular peak corresponding to the exact charge of the complex, without any axial ligand or counterion, could be only detected as the main peak in the case of the tricationic nickel derivative **3c-Ni** ($m/3$) and, as weaker peaks, in the cases of **4d-Mn** ($m/4$) and **8-Mn** ($m/5$, fig 2). For redox active manganese and iron complexes, we observed only the one-electron reduced form $m^-/3$ for the tetracationic complexes **2d-Mn**, **3d-Mn**, **3d-Fe**, **4d-Mn**, **5b-Mn**, **5c-Mn**, **5d-Mn** and $m^-/4$ for the pentacationic complex **8-Mn** (fig 2, on the left). This data suggested that Mn(III)- or Fe(III)-porphyrins were reduced in situ to the corresponding Mn(II) or Fe(II) derivatives when studied by ES-MS. Alternatively, the overall charge could be lowered by one unit more either by deprotonation, if the molecule contained a carboxylic acid group (eg, for **3d-Mn** and **3d-Fe** [m^- - $H^+/2$]) or by demethylation (eg, for **8-Mn** [m^- - $CH_3^+/4$]). In the case of **4d-Mn**, a manganese porphyrin with a fatty acid linker, we also observed a series of peaks at 420.8, 413.9, 406.8 and 399.7 which might result from a 1,4-loss of H_2 within the fatty acid chain, leading to a metalloporphyrin fragment with a terminal olefin (see fig 3). Such 1,4 loss of hydrogen was previously documented by Gross et al in FAB-MS studies of aliphatic fatty acids [25]. Taking in consideration this chain fragmentation and a two-electron reduction of the manganese(III) derivative leading to dicationic intermediates, the calculated peaks are respectively 420.9, 414.0, 407.0 and 399.9.

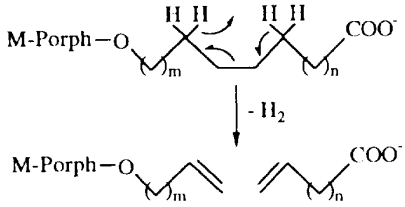

Fig 3. Cleavage of the side chain of **4d-Mn** observed by ES-MS.

Does the electrospray desorption method allow the determination of axial ligands or counterions?

In some cases, peaks were detected which seem to correspond with counteranion/pyridinium or extracounteranion/central metal ion-pairs suggesting that the stability of this tight-contact pair is compatible with the MS analysis conditions. Consequently, the corresponding charge was lowered by one unit (for Cl^-, I^-, OTs^-) or two units (for one SO_4^- or two OTs^-); the charge of tetracationic compounds was lowered to

Table II. Electrospray mass spectrometry data on porphyrin-HOE 33258 hybrid molecules.

Compound	*M*	*Formula*	*MW*[b]	*Molecular peak*[c]			*Other peak(s)*[c,d]			*Ref*
			m	m/z	*calc*	*obs*	m/z	*calc*	*obs*	*prep*
9	H_2	$C_{74}H_{64}N_{14}O_3$	1197.42		–		$m+4H^+/4$	300.4	300.4	*26*
							$m+3H^+/3$	400.1	400.2	
							$m+2H^+/2$	599.7	599.7	
10	H_2	$C_{78}H_{76}N_{14}O_3$	1257.56	$m+H^+/5$	251.7	251.7	$m+CH_3^+/5$	254.5	254.4	*26*
							$m+CH_3^+ +CH_2/5$	257.3	257.3	
				$m/4$	314.4	314.4	$m+CH_2/4$	317.9	318.0	
							$m+2CH_2/4$	321.4	321.3	
10-Mn	Mn^{III}, AcO^-	$C_{78}H_{74}N_{14}O_3Mn$	1310.50	$m/5$	262.1	262.3	$m+CH_2/5$	264.9	265.1	*26*
							$m+2CH_2/5$	267.7	267.9	
							$m+Cl^-/4$	336.5	336.3	
							$m+AcO^-/4$	338.9	338.9	
10-Ni[a]	Ni^{II}	$C_{78}H_{74}N_{14}O_3Ni$	1313.54	$m+H^+/5$	262.9	262.9	$m+CH_3^+/5$	265.7	265.7	*e*
							$m+CH_3^+ +CH_2/5$	268.6	268.6	
				$m/4$	328.4	328.4	$m+CH_2/4$	331.9	332.1	
							$m+2CH_2/4$	335.4	335.6	
10-Zn	Zn^{II}	$C_{78}H_{74}N_{14}O_3Zn$	1320.91	$m+H^+/5$	264.4	264.4	$m+CH_3^+/5$	267.2	267.1	*e*
							$m+CH_3^+ +CH_2/5$	270.0	270.0	
				$m/4$	330.2	330.3	$m+CH_2/4$	333.7	333.8	
							$m+2CH_2/4$	337.2	337.2	

[a] Masses were calculated using the mass of the main isotope of nickel (m = 58). [b] Calculated without the counterions and, in the case of metallated derivatives, without the axial ligands and the extra counterion if present. [c] Methylation of nitrogens of benzimidazole may result in either $+CH_3^+$ or $+CH_2$ when it is accompanied by a deprotonation on the other nitrogen. [d] The 100% peaks on the spectra were underlined. [e] This work.

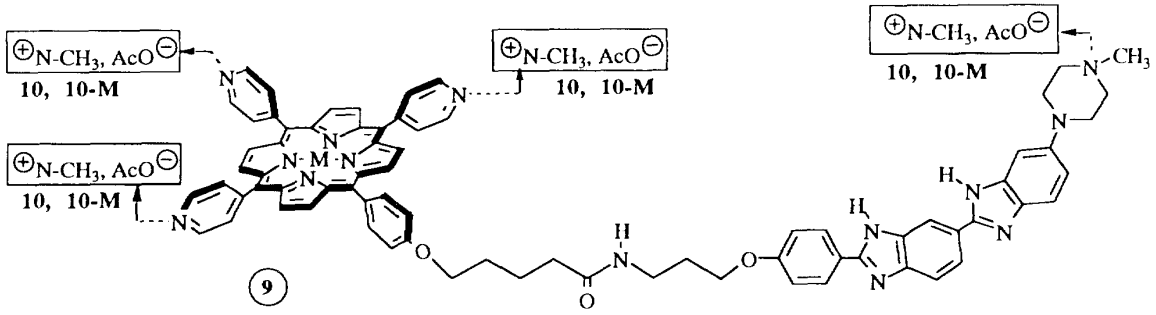

Fig 4. Structures of the metalloporphyrin-HOE 33258 hybrids **9**, **10** and **10-M** (M = Mn, Ni or Zn).

three in the case of **2d-Mn** or **3d-Mn** (m + $Cl^-/3$) and two for **3d-Fe** (m + $SO_4^{2-}/3$), **5b-Mn** and **5c-Mn** (m^- + $I^-/2$). For **7b** two peaks were observed at 302.5 and 539.4 which were due to the conservation of one (m + $OTs^-/3$) or two (m + $2OTs^-/2$) tosylate counterions, respectively. So, in some cases, a careful interpretation of ES-MS spectra might confirm the nature of counterions present in cationic porphyrin derivatives.

Case of 'porphyrin-HOE 33258' hybrid molecules

The hybrid molecule **9** is prepared by coupling the porphyrin derivative **3a** with HOE 33258, a dye known for its binding in the minor groove of B-DNA with high affinity for AT rich regions [26]. This hydrophobic compound was methylated (compound **10**) and then metallated with Mn, Ni or Zn to give **10-Mn**, **10-Ni**, **10-Zn**, respectively (see ref [27] for preparation of **9**, **10** and **10-Mn**). ES-MS data are listed in table II. In the case of the hydrophobic compound **9**, which could be easily protonated, the main peak (m + $3H^+/3$) corresponded to the trication. We also observed two (m + $2H^+/2$) and four (m + $4H^+/4$) charged species. Protonation probably occurs on two or three of the pyridines of the porphyrin, and/or the piperazine and the benzimidazole of HOE 33258.

For the four tetracationic products **10**, **10-Mn**, **10-Ni** and **10-Zn**, two main groups of peaks were observed: four and five charged 'families' (see the case of **10-Ni** on the right of fig 2). In each group, we observed the molecular peaks $m/5$ (**10-Mn**), $m/4$ and their protonated forms m + $H^+/5$ (**10**, **10-Ni**, **10-Zn**). Other peaks correspond to *trans*-methylated compounds. Two types of addition were observed: either methylation on N^3 of a benzimidazole ring is followed by the loss of the proton of N^1 with an increase in mass units corresponding to CH_2 (the charge was unaffected), or, in the absence of deprotonation, the mass increase cor-

responded to CH_3 (and charge increased of one unit). Generally one ($+CH_2$ or $+CH_3^+$) or two ($+CH_3^+ + CH_2$ or $+2CH_2$) methylations were observed simultaneously giving, with the protonated forms and the molecular ones, a characteristic profile for pentacationic and tetracationic families (fig 2, on the right).

Experimental section

General methods

Proton NMR spectra were recorded at 200, 250 or 400 MHz in the indicated solvent, and shifts are reported in ppm. DCI-MS (DCI-NH_3) and FAB-MS (glycerol) analyses were performed on a Nermag R1010 at the MS-Service of the Chemistry Department of the Université Paul-Sabatier, Toulouse. ES-MS analyses were performed on a VG Trio 2000 at the Laboratory of Pharmacology, CNRS, Toulouse (solvent and eluent were H_2O/CH_3CN, 1:1).

Chemicals

All reagents or compounds not explicitly referenced were obtained from commercial sources and used as received. DMF was distilled under reduced pressure. TLC was performed using Merck silica-gel $60F_{254}$ precoated plates, 0.2 mm thickness. Column chromatography was carried out using silica gel (70-230 mesh, SDS).

Preparation of the unsymmetrical tripyridylporphyrin precursor **1**: *{5-[4-(1-oxopropoxy)phenyl]-10,15,20-tris(4-pyridyl)}porphyrin*

The synthesis of the unsymmetrical porphyrin with three pyridyl groups and a phenyl substituent with a *p*-hydroxyl function protected by a propanoyl moiety was carried out in propanoic acid as previously described [12]. A mixture of 4-hydroxybenzaldehyde (3.646 g, 0.03 mol), propanoic acid (234 mL) and acetic anhydride (12 mL) was heated at 140 °C with stirring. To this solution were successively and slowly added 4-pyridinecarboxaldehyde (8.5 mL, 0.097 mol) and pyrrole (8.5 mL, 0.115 mol). The resulting mixture was refluxed for 1.5 h. The volume of solvent was reduced to 50 mL under reduced pressure. The mixture was then neutralized with an aqueous $KHCO_3$ solution, filtered through cotton, washed with water and dried under reduced pressure. Purification was modified and performed as follows: crude material was dissolved in CH_2Cl_2 (350 mL), hexane (290 mL) was then added and the mixture was allowed to precipitate overnight at 4 °C. The supernatant was removed and dried under reduced pressure. The resulting solid was dissolved in ethanol (150 mL) with CH_2Cl_2 (20 mL), hexane was added (150 mL) and the mixture was allowed to precipitate overnight at 4 °C. The supernatant was drawn off, the precipitate was dissolved in a CH_2Cl_2/EtOH mixture (96:4, v/v) and purified by silica-gel column chromatography (eluent was CH_2Cl_2/EtOH, 96:4, v/v). Evaporation of solvent afforded 400 mg of the desired compound **1** as a purple powder. Analytical data were as reported in the literature [12].

Preparation of {5-[4-(carboxymethoxy)phenyl]-10,15,20-tris(4-pyridyl)}porphyrin **2a** *and {5-[4-[(11-carboxyundecyl)oxy]phenyl]-10,15,20-tris(4-pyridyl)}porphyrin* **4a**

To a solution of **1** (150 mg, 0.22 mmol) in dry DMF (15 mL) under nitrogen was added powdered sodium hydroxide (225 mg, 5 mmol), and the mixture was stirred at room temperature for 30 min. Formation of the phenolate form was checked by TLC before addition of iodoacetic acid (80 mg, 0.43 mmol) for **2a** or 12-bromododecanoic acid (120 mg, 0.43 mmol) for **4a**. The mixture was stirred for 2 h at room temperature (**2a**) or at 65 °C (**4a**), and neutralized with a 2% aqueous citric acid solution. The crude material was then extracted twice with dichloromethane. The organic extracts were combinated, dried over sodium sulfate and evaporated to dryness. Products were purified on silica with a CH_2Cl_2/EtOH (96:4, v/v) eluent and precipitated from a dichloromethane/hexane mixture. Yields were 80% (**2a**) and 75% (**4a**).

Data for **2a**: UV-vis (CH_2Cl_2): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 416 nm (38×10^4).

^1H NMR (TFA): δ = 9.45 (m, 6H, 2,6-pyridine), 9.03 (m, 6H, 3,5-pyridine), 8.79 (m, 4H, β-pyrrole), 8.61 (m, 4H, β-pyrrole), 8.35 (d, 2H, J = 7.7 Hz, 2,6-phenyl), 7.43 (d, 2H, J = 7.7 Hz, 3,5-phenyl), 4.95 (s, 2H, CH_2).

MS (DCI, NH_3) m/z 693 ($M^+ + 1$).

Data for **4a**. UV-vis (CH_2Cl_2): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 416 nm (38×10^4).

^1H NMR (TFA): δ = 9.32 (m, 6H, 2,6-pyridine), 9.02 (m, 6H, 3,5-pyridine), 8.80 (m, 2H, β-pyrrole), 8.7 (m, 2H, β-pyrrole), 8.58 (m, 4H, β-pyrrole), 8.35 (d, 2H, J = 7.9 Hz, 2,6-phenyl), 7.52 (d, 2H, J = 8 Hz, 3,5-phenyl), 4.22 (t, 2H, J = 5.6 Hz, -$PhOCH_2$), 2.45 (t, 2H, J = 6.4 Hz, -CH_2COO-), 1.80 (m, 2H, CH_2), 1.47 (m, 4H, 2 CH_2), 1.15 (m, 12H, 6 CH_2).

MS (FAB) m/z 832 (M^+).

Preparation of {5-[4-[(ethoxycarbonyl)methoxy]phenyl]-10,15,20-tris(4-pyridyl)}porphyrin **2b** *and {5-[4-[11-(ethoxycarbonyl)undecyloxy]phenyl]-10,15,20-tris(4-pyridyl)}porphyrin* **4b**

Esterification of **2a** and **4a** led to porphyrins **2b** and **4b**, respectively. The same procedure was used in both cases. After dissolving **2a** (40 mg, 48 μmol) in ethanol (12 mL), sulfuric acid (50 μL, 0.93 mmol) was added and the mixture was refluxed for 1.5 h. Formation of the ester was checked by TLC. The volume was extended to 100 mL with water, and the mixture was neutralized with a NaOH solution. The aqueous layer was washed several times with dichloromethane, until the organic extracts were colorless. These organic extracts were then dried over sodium sulfate and evaporated to dryness. Products were purified on silica with a CH_2Cl_2/EtOH (96:4, v/v) eluent. Compounds **2b** and **4b** were recovered by evaporation of the solvent in 70% and 64% yields.

Data for **2b**: UV-vis (CH_2Cl_2): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 416 nm (39×10^4).

^1H NMR (CD_2Cl_2): δ = 9.02 (d, 6H, J = 5.5 Hz, 2,6-pyridine), 8.96 (d, 2H, J = 5 Hz, β-pyrrole), 8.88 (s, 4H, β-pyrrole), 8.85 (d, 2H, J = 5 Hz, β-pyrrole), 8.15 (d, 6H, J = 5.6 Hz, 3,5-pyridine), 8.10 (d, 2H, J = 8.6 Hz, 2,6-phenyl), 7.35 (d, 2H, J = 8.6 Hz, 3,5-phenyl), 4,95 (s, 2H, CH_2), 4.42 (q, 2H, J = 7 Hz, -$COOCH_2$), 1.40 (t, 3H, J = 7 Hz, -CH_3), −2.88 (s, 2H, NH pyrrole).

MS (DCI, NH_3), m/z 721 ($M^+ + 1$).

Data for **4b**: UV-vis (CH_2Cl_2): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 416 nm (37×10^4).

^1H NMR (TFA): δ = 9.32 (m, 6H, 2,6-pyridine), 9.02 (m, 6H, 3,5-pyridine), 8.85 (m, 2H, β-pyrrole), 8.78 (m, 2H, β-pyrrole), 8.57 (m, 4H, β-pyrrole), 8.35 (d, 2H, J = 7.9 Hz, 2,6-phenyl), 7.56 (d, 2H, J = 8 Hz, 3,5-phenyl), 4.25 (t, 2H, J = 5.3 Hz, -$PhOCH_2$), 4.02 (q, 2H, J = 5.8 Hz, $COOCH_2$), 2.21 (t, 2H, J = 6.8 Hz, -CH_2COO-), 1.85 (m, 2H, CH_2), 1.47 (m, 4H, 2 CH_2), 1.15 (m, 12H, 7 CH_2 + 3H, CH_3).

MS (FAB): m/z 860 (M^+).

General procedure for methylation of porphyrins

Porphyrins **2b**, **4b** and **5a** were methylated in DMF with a large excess of methyl iodide (100 equiv) for 3 h at room temperature, as previously described [12]. The reaction was monitored by TLC with a mixture CH_2Cl_2/EtOH (95:5, v/v) as eluent (the final products do not migrate). The excess of methyl iodide was removed under vacuum at room temperature and DMF by heating at 100 °C under reduced pressure. These reactions were quantitative and led to **2c**, **4c** and **5b**, respectively.

- *{5-[4-[(Ethoxycarbonyl)methoxy]phenyl]-10,15,20-tris(1-methylpyridinium-4-yl)}porphyrin* **2c**

UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 426 nm (18×10^4).

^1H NMR (DMSO): δ = 9.60 (d, 6H, J = 6.1 Hz, 2,6-pyridinium), 9.26 (s, 4H, β-pyrrole), 9.17 (s, 4H, β-pyrrole), 9.12 (d, 6H, J = 6.3 Hz, 3,5-pyridinium), 8.26 (d, 2H, J = 7.9 Hz, 2,6-phenyl), 7.56 (d, 2H, J = 8 Hz, 3,5-phenyl), 5.25 (s, 2H, -OCH_2COO-), 4.83 (s, 9H, N^+-CH_3), 4.46 (q, 2H, J = 7.1 Hz, -$COOCH_2$), 1.45 (t, 3H, J = 7 Hz, -CH_3), −2.92 (s, 2H, NH pyrrole).

- *{5-[4-[11-(Ethoxycarbonyl)undecyloxy]phenyl]-10,15,20-tris(1-methylpyridinium-4-yl)} porphyrin* **4c**

UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 424 nm (18×10^4).

^1H NMR (DMSO): δ = 9.59 (d, 6H, J = 6.3 Hz, 2,6-pyridinium), 9.51 (s, 4H, β-pyrrole), 9.17 (s, 4H, β-pyrrole), 9.12 (d, 6H, J = 6.5 Hz, 3,5-pyridinium), 8.25 (d, 2H, J = 8.8 Hz, 2,6-phenyl), 7.54 (d, 2H, J = 8.8 Hz, 3,5-phenyl), 4.83 (s, 9H, N^+-CH_3), 4.40 (t, 2H, J = 5.6 Hz, -$PhOCH_2$), 4.15 (q, 2H, J = 7 Hz, -$COOCH_2$), 2.40 (t, 2H, J = 7.5 Hz, -CH_2COO-), 2.02 (m, 2H, CH_2), 1.39 (m, 16H, 8 CH_2), 1.28 (t, 3H, J = 7 Hz, -CH_3), −2.97 (s, 2H, NH pyrrole).

- *{5-[4-[3-(Tritylamino)propyloxy]phenyl]-10,15,20-tris(1-methylpyridinium-4-yl)}porphyrin* **5b**

UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 426 nm (19×10^4).

^1H NMR (DMSO): 9.67 (d, 6H, J = 6.3 Hz, 2,6-pyridinium), 9.51 (s, 4H, β-pyrrole), 9.15 (s, 4H, β-pyrrole), 9.12 (d, 6H, J = 6.2 Hz, 3,5-pyridinium), 8.27 (d, 2H, J = 8.7 Hz, 2,6-phenyl), 7.52 (m, 15H, trityl + 2H, 3,5-phenyl), 4.84 (s, 9H, -N^+-CH_3), 4.58 (t, 2H, -$PhOCH_2$-), ≈ 2.55 (4H, 2 CH_2, under DMSO), −2.92 (s, 2H, NH pyrrole).

Preparation of {5-[4-[carboxymethoxy)phenyl]-10,15,20-tris(1-methylpyridinium-4-yl)}porphyrin **2d** *and {5-[4-[(11-carboxyundecyl)oxy]phenyl]-10,15,20-tris(1-methylpyridinium-4-yl)}porphyrin* **4d**

Porphyrin **2c** (or **4c**) was dissolved in a 6 M aqueous hydrochloric acid solution (10 mL), and the mixture was stirred for 3 h at room temperature. Water was then removed under reduced pressure until dryness. The resulting material was dissolved in pure water, and the mixture was filtered through a glass frit to eliminate the unreacted ester. Compound **2d** (or **4d**) was recovered by drying the filtrate under reduced pressure. The yield of this reaction was 80% in both cases.

Data for **2d**: UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 424 nm (17×10^4).

^1H NMR (DMSO): δ = 9.61 (d, 6H, J = 6.2 Hz, 2,6-pyridinium), 9.20 (s, 4H, β-pyrrole), 9.17 (s, 4H, β-pyrrole), 9.12 (d, 6H, J = 7 Hz, 3,5-pyridinium), 8.26 (d, 2H, J = 8.7 Hz, 2,6-phenyl), 7.55 (d, 2H, J = 8.7 Hz, 3,5-phenyl), 5.12 (s, 2H, -OCH_2COO-), 4.84 (s, 9H, N^+-CH_3), −2.90 (s, 2H, NH pyrrole).

Data for **4d**: UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 426 nm (18×10^4).

^1H NMR (DMSO): δ = 9.60 (d, 6H, J = 6 Hz, 2,6-pyridinium), 9.26 (s, 4H, β-pyrrole), 9.17 (s, 4H, β-pyrrole), 9.12 (d, 6H, J = 6.6 Hz, 3,5-pyridinium), 8.25 (d, 2H, J = 8.6 Hz, 2,6-phenyl), 7.55 (d, 2H, J = 8.6 Hz, 3,5-phenyl), 4.84 (s, 9H, N^+-CH_3), 4.40 (t, 2H, J = 5.6 Hz, -$PhOCH_2$), 2.32 (t, 2H, J = 7.2 Hz, -CH_2COO-), 2.02 (m, 2H, CH_2), 1.43 (m, 16H, 8 CH_2), −2.90 (s, 2H, NH pyrrole).

Preparation of {5-[4-[(3-ammoniopropyl)oxy]phenyl]-10,15,20-tris(1-methylpyridinium-4-yl)} porphyrin benzotriazolate **5c** *and its manganese derivative* **5c-Mn**

Free amine functions of **5c** and **5c-Mn** are recovered by deprotection of **5b** and **5b-Mn**, respectively. The same procedure was used in both cases. Compound **5b** (150 mg, 110 μmol) was dissolved in a 0.5 M solution of 1-hydroxybenzotriazole in trifluoroethanol (15 mL). The mixture was stirred at room temperature for 4 h. Diethyl ether was then added, and the mixture was centrifuged. The pellet was dissolved in trifluoroethanol and the excess of 1-hydroxybenzotriazole was eliminated by precipitation with diethyl ether. **5c** was recovered as a black powder by drying the pellet. Yields were 98% (**5c**) and 96% (**5c-Mn**).

Data for **5c**: UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 426 nm (19×10^4).

^1H NMR (DMSO): δ = 9.60 (d, 6H, J = 6.3 Hz, 2,6-pyridinium), 9.27 (s, 4H, β-pyrrole), 9.15 (m, 4H, β-pyrrole + 6H, 3,5-pyridinium), 8.29 (d, 2H, J = 8.8 Hz, 2,6-phenyl), 7.56 (d, 2H, J = 8.5 Hz, 3,5-phenyl), 4.83 (s, 9H, N^+-CH_3), 4.50 (t, 2H, J = 4.9 Hz, -$PhOCH_2$), 3.30 (t, 2H, J = 7 Hz, -CH_2N-), 2.35 (m, 2H, CH_2), −2.90 (s, 2H, NH pyrrole).

Data for **5c-Mn**: UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 464 nm (10^5).

Preparation of {5-[4-[3-(iodoacetamido)propyloxy]-phenyl]-10,15,20-tris(1-methylpyridinium-4-yl)} porphyrin **5d** *and its manganese derivative* **5d-Mn**

These compounds were prepared from **5c** and **5c-Mn**, respectively, using the same procedure in both cases. **5c** (70 mg, 56 μmol) was dissolved in anhydrous DMF (10 mL), and the mixture was cooled to 0 °C. A cold solution (0 °C) of iodoacetic anhydride (60 mg, 170 μmol) in anhydrous DMF (5 mL) was then added. The resulting mixture was stirred for 2 h at 0 °C, and diethylether was added. After precipitation, the mixture was centrifuged and another precipitation (in a DMF/diethyl ether mixture) was performed to eliminate the excess of iodoacetic anhydride. After centrifugation, the pellet was dried under vacuum at room temperature. Yield = 95%.

Data for **5d**: UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 428 nm (20×10^4).

^1H NMR (DMSO): δ = 9.66 (d, 6H, J = 6.2 Hz, 2,6-pyridinium), 9.49 (s, 4H, β-pyrrole), 9.17 (s, 4H, β-pyrrole), 9.12 (d, 6H, J = 6.4 Hz, 3.5-pyridinium), 8.61 (broad s, 1H, -NH-), 8.27 (d, 2H, J = 8.5 Hz, 2,6-phenyl), 7.56 (d, 2H, J = 8.5 Hz, 3,5-phenyl), 4.89 (s, 9H, N^+-CH_3), 4.44 (t, 2H, -$PhOCH_2$), 3.83 (s, 2H, -$COCH_2I$), ≈3.30 (2H, -CH_2N-, under H_2O), 2.35 (m, 2H, CH_2), 2.20 (m, 2H, -CH_2-), −2.90 (s, 2H, NH pyrrole).

Data for **5d-Mn**: UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 464 nm (9.7×10^4).

• *Preparation of* **5e**

After dissolving **5d** (25 mg, 19 μmol) in 100 mM Tris-HCl buffer, pH 8 (15 mL), glutathione (60 mg, 195 μmol) was added and the mixture was stirred for 4 h at room temperature. The mixture was then precipitated by adding acetone. The pellet was recovered by centrifugation and was dried under reduced pressure. The yield was 42%.

• *Metallation of* **2d** *and* **4d** *with* $MnCl_2$

These compounds were metallated following the same procedure. Compound **2d** (200 mg, 160 μmol) was dissolved in water (40 mL), and the mixture was heated to reflux. Manganese chloride (250 mg, 1.76 mmol) was then added, and the mixture was stirred for 1.5 h. Metallation of the porphyrin was monitored by UV-vis. The mixture was cooled to room temperature and DMF (140 mL) and THF (280 mL) were added. The mixture was allowed to precipitate overnight at 4 °C, the pellet was recovered after centrifugation and was dried under reduced pressure. Yield for **2d-Mn** = 90%, yield for **4d-Mn** = 84%.

2d-Mn: UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 464 nm (10^5). **4d-Mn**: UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 466 nm (8.5×10^4).

• *Metallation of* **3d** *with ammonium iron(II) sulfate*

Compound **3d** was metallated with $(NH_4)_2Fe(SO_4)_2$ following the same procedure. The excess of $(NH_4)_2Fe(SO_4)_2$ was eliminated by precipitation in methanol (only the metallated porphyrin was soluble in methanol). Compound **3d-Fe** was recovered by evaporating the solvent under reduced pressure and was obtained in a 80% yield.

UV-vis (H_2O): λ_{max} (ε, $M^{-1} \times cm^{-1}$) 424 nm (9.1×10^4).

Preparation of methyl 6-formylnicotinate **6**

Methyl 6-methylnicotinate (2 g, 13 mmol), iodine (3.4 g, 13 mmol), trifluoroacetic acid (3 mL, 39 mmol) and *tert*-butyl iodide (620 μL, 5.2 mmol) were dissolved in 50 mL dry DMSO and the mixture was refluxed for 3 h. The reaction was followed by TLC (CH_2Cl_2/EtOH 97:3) with 2,4-dinitrophenylhydrazine detection. The solution was diluted with 0.1 N $Na_2S_2O_3$, and 10% $NaHCO_3$ water solution was added until basicity. The aqueous layer was extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and evaporated to dryness. Purification on silica with a CH_2Cl_2/EtOH (97:3) eluent gave methyl 6-formylnicotinate in 57% yield.

UV-vis (59 μM in CH_2Cl_2) λ_{max} (ε, $M^{-1} \times cm^{-1}$) 242 (14×10^3), 248 nm (6×10^3).

^{1}H NMR (CD_2Cl_2) δ = 10.12 (d, 1H, J^5 = 0.7 Hz, CHO), 9.35 (dd, 1H, J^4 = 2 Hz, J^5 = 0.7 Hz, H_2), 8.47 (ddd, 1H, J = 8 Hz, J^4 = 2 Hz, J^5 = 0.7 Hz, H_4), 8.03 (dd, 1H, J = 8 Hz, J^5 = 0.7 Hz, H_5), 4.00 (s, 3H, $COOCH_3$).

MS-DCI m/z 166 ($M^+ + 1$), 183 ($M^+ + 18$).

Anal calc for $C_8H_7NO_3$: C 58.18, H 4.27, N 8.48; found: C 57.55, H 4.20, N 8.24.

Preparation of {5-[5-(methoxycarbonyl)-2-pyridyl]-10,15,20-tris(4-pyridyl)}porphyrin **7a**

A mixture of methyl 6-formylnicotinate **6** (3.8 g, 22 mmol), propanoic acid (66 mL) and acetic anhydride (4 mL) was heated at 140 °C with stirring. To this solution were successively and slowly added 4-pyridinecarboxaldehyde (3.7 mL, 50 mmol) and pyrrole (3.32 mL, 38 mmol). The resulting mixture was refluxed for 1.5 h. The mixture was then neutralized with $KHCO_3$, filtered through glass frit and washed several times with water. The crude material was dissolved in CH_2Cl_2 and precipitated with hexane (30:70). The supernatant was dried and purified by column chromatography on silica gel with a CH_2Cl_2/EtOH (96:4) eluent. The product was precipitated by addition of hexane to a dichloromethane solution (yield = 1%).

UV-vis (1.9 μM in CH_2Cl_2) λ_{max} (ε, $M^{-1} \times cm^{-1}$) 588 (5×10^3), 512 (16×10^3), 418 nm (33×10^4, Soret band).

^{1}H NMR (CD_2Cl_2) δ = 9.72 (dd, 1H, J^4 = 2 Hz, J^5 = 0.6 Hz, H'_6), 9.04 (d, 6H, J = 6 Hz, 2,6-pyridine), 8.93 (s, 8H, β-pyrrole), 8.74 (dd, 1H, J = 8 Hz, J^4 = 2 Hz, H'_4), 8.38 (dd, 1H, J = 8 Hz, J^5 = 0.6 Hz, H'_3), 8.18 (d, 6H, J = 6 Hz, 3,5-pyridine), 4.15 (s, 3H, $COOCH_3$), −2.92 (s, 2H, NH pyrrole).

MS-DCI m/z 677 ($M^+ + 1$).

Anal calc for $C_{42}H_{28}N_8O_2$: C 74.54, H 4.17, N 16.56; found: C 72.64, H 4.00, N 15.92.

• *{5-[5-(Methoxycarbonyl)-1-methylpyridinium-2-yl]-10,15,20-tris(1-methylpyridinium-4-yl)} porphyrin tetratosylate* **7b**

Porphyrin **7a** was methylated in DMF with 30 equiv of methyl *p*-toluenesulfonate by refluxing for 2 h. The solution was diluted with water. The aqueous layer was washed three times with CH_2Cl_2 and dried under vacuum. The product was precipitated by addition of acetone to a methanol solution. This reaction was quantitative.

UV-vis (3.2 μM in H_2O) λ_{max} (ε, $M^{-1} \times cm^{-1}$) 586 (6×10^3), 518 (13×10^3), 422 nm (18×10^4, Soret band).

^{1}H NMR (DMSO) δ = 10.38 (s, 1H, H'_6), 9.56 (m, 7H, 2,6-pyridinium and H'_4), 9.37 (m, 8H, β-pyrrole), 9.11 (m, 7H, 3,5-pyridinium and H'_3), 7.56 (d, 8H, J = 8 Hz, 2,6-tosylate), 7.19 (d, 8H, J = 8 Hz, 3,5-tosylate), 4.84 (s, 9H, N^+-Me), 4.33 (s, 3H, $COOCH_3$), 4.26 (s, 3H, N^+-Me), 2.36 (s, 12H, CH_3-tosylate), −2.95 (s, 2H, NH pyrrole).

• *5-(5-Carboxy-1-methylpyridinium-2-yl)-10,15,20-tris(1-methylpyridinium-4-yl)porphyrin tetratosylate* **7c**

Porphyrin **7b** (100 mg, 70 μmol) was deprotected by stirring in a 6 M HCl solution (30 mL) for 6 h at 75 °C. Methanol resulting from ester hydrolysis was eliminated during the reaction using a gentle stream of nitrogen. Yield = 98%.

UV-vis (4.2 μM in H_2O) λ_{max} (ε, $M^{-1} \times cm^{-1}$) 584 (5×10^3), 516 (11×10^3), 420 nm (16×10^4, Soret band).

^{1}H NMR (DMSO) δ = 10.33 (s, 1H, H'_6), 9.62 (d, 6H, J = 6 Hz, 2,6-pyridinium), 9.50 (d, 1H, J = 8 Hz, H'_4), 9.37 (m, 8H, β-pyrrole), 9.12 (m, 7H, 3,5-pyridinium and H'_3), 7.57 (d, 8H, J = 8 Hz, 2,6-tosylate), 7.23 (d, 8H, J = 8 Hz, 3,5-tosylate), 4.84 (s, 9H, N^+-Me), 4.26 (s, 3H, N^+-Me), 2.37 (s, 12H, CH_3-tosylate), −2.95 (s, 2H, NH pyrrole).

Preparation of cationic 'metalloporphyrin-HOE 33258' hybrid molecules **10-Ni** *and* **10-Zn**

Synthesis of the 'porphyrin-HOE 33258' hybrid precursor **9**, the tetracationic derivative **10** and its manganese derivative **10-Mn** were carried out according to a described method [27]. Metallation with Ni or Zn was performed as follows: to a 600 μL DMF solution of **10** (9 mg, 6 μmol) at 100 °C were added 7 mg (30 μmol) of $NiCl_2 \cdot 6H_2O$ or 7 mg (30 μmol) of $Zn(AcO)_2 \cdot 2H_2O$ and 6.5 μL (50 mmol) of 2,4,6-collidine. The reaction was completed after 3 h. The DMF solution was extended to 200 μL with water, and the metallated molecules were precipitated by adding 2.4 mL THF.

Ion-exchange chromatography on a Bio-Rad resine (acetate form) gave **10-Ni** and **10-Zn** in a 85% yield. Purity of each cationic product was checked by HPLC on a 10 μ C_{18} Ultrabase column (Société française de chromatographie). The eluent was a linear gradient of acetonitrile and 0.1 M AcONa, pH 3 (10:90 to 90:10, v/v, in 30 min). Peaks corresponding to hybrid molecules showed both the absorptions at 340 nm for HOE 33258 moiety and at 430 nm (**9** and **10-Ni**), 468 nm (**10-Mn**) or 440 nm (**10-Zn**) for the porphyrin part. Comparative retention times were as follows: HOE 33258, 12 min; **10**, 14 min; **10-Mn**, 17 min; **10-Ni**, 17.5 min; **10-Zn**, 16.5 min.

Data: **10-Ni**: UV-vis (5 μM in H_2O) λ_{max} (ε, $M^{-1} \times cm^{-1}$) 260 (2×10^4), 342 (2.5×10^4), 430 (5×10^4, Soret band), 538 nm (4×10^3). **10-Zn**: UV-vis (5 μM in H_2O) λ_{max} (ε, $M^{-1} \times cm^{-1}$) 260 (4×10^4), 340 (5×10^4), 448 (10×10^4, Soret band), 570 (1×10^4), 616 nm (6×10^3).

Preparations of **3a**, **3b**, **3c**, **3c-Ni**, **3d**, **3d-Mn**, **5a** and **5b-Mn** were described in ref [12].

Conclusion

Several tricationic metalloporphyrin derivatives known for their oxidative DNA cleavage capacity were synthesized with different functionalized tethers (acid, amine or iodoacetamide functions) in order to link such DNA cleavers to oligonucleotides or minor groove binders. To illustrate the latter, hybrid molecules containing a cationic porphyrin linked to Hoechst 33258 were prepared. All these cationic porphyrin derivatives were characterized by electrospray mass spectrometry, the only method adapted to the mass determination of these non-volatile charged porphyrin derivatives. Nearly all major peaks of ES-MS spectra can be interpreted taking in account several factors: the possible deprotonation of functionalized tethers or pyrrolic N-H, the one-electron reduction of the non-metallated porphyrin macrocycle, the one- or two-electron reduction of metalloporphyrins, and the occurrence in some cases of fragmentations and demethylation or transmethylation reactions.

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