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SYNTHESIS OF HYBRID "METALLOPORPHYRIN-ELLIPTICINE" MOLECULES

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The synthesis of hybrid "metalloporphyrin-ellipticine" molecules is reported. These molecules are based on 9-methoxyellipticine and [5-(4-hydroxyphenyl)-10,15,20-tris(4-tolyl)]porphyrin linked by a ten-bond chain, -$(CH_2)_4$-CO-NH-$(CH_2)_3$-, between the hydroxyl group of the porphyrin and the pyridine nitrogen of the pyrido-carbazole. These molecules have been metallated with manganese, iron or zinc salts.

La synthèse de molécules hybrides "métalloporphyrine-ellipticine" est décrite. La structure de ces molécules est basée sur la 9-méthoxyellipticine et la [5-(hydroxy-4-phényl)-10,15,20-tris(tolyl-4)]porphyrine liées par un bras à dix chaînons, -$(CH_2)_4$-CO-NH-$(CH_2)_3$-, entre le groupement hydroxyle de la porphyrine et l'azote pyridinique du pyrido-carbazole. Ces molécules ont été métallées avec le manganèse, le fer ou le zinc.

Introduction

Among all antitumor agents, bleomycin has to be regarded as a particular case since its biological activity results from DNA cleavage generated in the presence of three co-factors: iron or copper salts, molecular oxygen and an electron source.[1]

The DNA cleavage mainly occurs via single-strand breaks due to the abstraction of an hydrogen atom at a C'_4 position of deoxyribose ring by an high-valent metal-oxo species chelated by the bleomycin (see review articles 1a and 1b on the sugar ring oxidation leading to DNA cleavage, and references 1f-h for discussions on bleomycin-iron-oxo species as reactive intermediate).

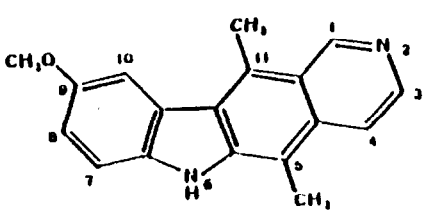
Bleomycin can be considered as the paradigm molecule for DNA cleavers with its structural duality: one part of the molecule is responsible for the DNA interaction, namely the bi-thiazole moiety, whereas a second part is a strong chelator for metal ions. Based on this duality, artificial DNA cleavers have been synthesized during the last six years by associating a metalloporphyrin[2] or an EDTA moiety[3] with an intercalating agent.

Since our group is involved in the oxygenation of hydrocarbons by metalloporphyrins,[4] by the molecular pharmacology of ellipticine derivatives[5] and by DNA cleavage with a water-soluble manganese-porphyrin complex and potassium monopersulphate,[6] we report here the synthesis of manganese-, iron- and zinc-porphyrins linked to 9-methoxyellipticine.[7] We will also present the experiments performed in order to test the ability of these molecules as DNA cleavers.

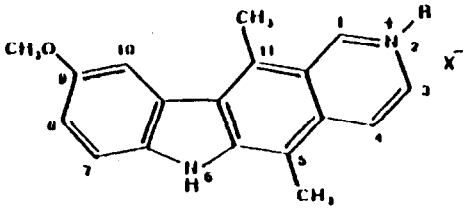
Results and Discussion

Chemical Syntheses of Hybrid "Metalloporphyrin-Ellipticine" Molecules. The ellipticine derivative used in these syntheses of hybrid molecules is 9-methoxyellipticine, an ellipticine derivative which can be easily prepared from 5-methoxyindole[8] according Dalton *et al.* or by other more recent published methods.[9] Some clinical trials on its use as antitumor agent have been reported[10] but it is another ellipticine derivative, *viz.* ellipticinium acetate, which is currently used in cancer chemotherapy.[11]

9-methoxyellipticine (1) (Figure 1) was alkylated at the pyridine nitrogen by 5-bromovaleric acid ethyl ester in dimethylformamide for 4 h at 120 °C. The expected product (2) was obtained as an orange powder in 87% yield after precipitation by diethylether.



1 9-methoxyellipticine



2 R = -CH_2-CH_2-CH_2-CH_2-CO_2-CH_2-CH_3 X = Br

3 R = -$(CH_2)_4$-CO_2H X = Cl^- or AcO^-

3bis R = -$(CH_2)_4$-CO-O-CO-OEt

Figure 1- Structure of 9-methoxyellipticine derivatives.

After treatment by HCl 1M of (2) under reflux for 3 h, the corresponding derivative (3) with a free acid function was quantitatively recovered. The counter-anion, Cl^-, can be exchanged to an acetate by ion-exchange resin, prealably washed by an acetic acid solution. In that case, the molecular peak (M^+ = 377) can be observed by desorption-chemical ionization mass spectrometry. The strategy for the synthesis of hybrid molecules was based on the mixed anhydride method.[12] (6) is obtained by the coupling of the mixed anhydride formed from (3) and ethyl chloroformate with metal-free porphyrin bearing an amino function attached by a methylene chain to the para-phenoxy ring of a meso-tetraarylporphyrin. This class of synthetic porphyrin is known to be more resistant to oxidation conditions than natural porphyrins without substituents at meso positions.[4c]

The basic porphyrin, [5-(4-hydroxyphenyl)-10,15,20-tris(4-tolyl)]porphyrin, (4) (Figure 2), has been prepared according to a modified procedure published by Little *et al.*[13] A propionic acid solution of two equivalents of *p*-tolylaldehyde, one equivalent of *p*-hydroxybenzaldehyde and three equivalents of pyrrole was refluxed for one hour. This molecular ratio of the three components has been chosen after several trials and leads to the best yield of the desired [5-(4-hydroxyphenyl)-10,15,20-tris(4-tolyl)]porphyrin derivative. From a crude porphyrin material obtained in 9% yield, the desired derivative (4) was obtained in an overall 6% yield after purification and separation by chromatography on a silica gel column. (4) was etherified with 3-bromopropylamine in dimethylformamide in the presence of sodium hydroxide (fine powder obtained from pellets). After extraction by dichloromethane and

purification by chromatography on a silica gel column, the porphyrin (5) with an amino function was obtained in 87% yield.

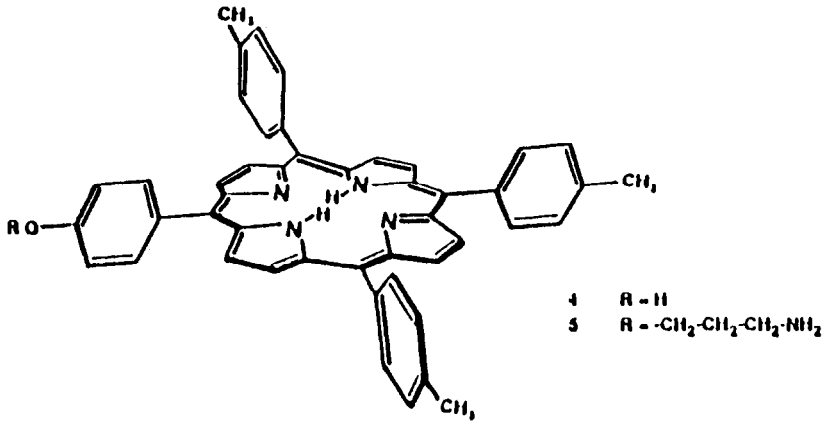


Figure 2- Structure of porphyrin precursors.

The hybrid molecule (6) (Figure 3) was obtained by coupling of both ellipticine and porphyrin precursors after generating the mixed anhydride (3bis) by treatment of (3) with ethylchloroformate in dichloromethane in the presence of triethylamine and evaporation to dryness to remove all traces of ethylchloroformate. The amino-porphyrin (5) then was added to a dichloromethane solution of (3bis) containing triethylamine as acid trap. After two hours at refluxing temperature and one extra hour at room temperature, the coupling reaction was complete. After purification by preparative thin-layer chromatography avoiding direct irradiation by light (protection of glassware by aluminium foils), the pure hybrid molecule (6) was obtained in 47% yield (exposure of solutions of (6) to light for hours leads to an important degradation of the hybrid molecule, which has been attributed to the reaction of (6) with singlet oxygen, since non-metallated porphyrin are photoactivable molecules and also because preliminary ^1H n.m.r. studies indicate that the ellipticine moiety is probably strongly stacked above the porphyrin ring). Both field-desorption and fast-atom bombardment mass spectrometries of (6) gave a peak at 1088 (M^+-H) indicating that the coupling of both parts of the hybrid molecule had occurred. u.v.-visible and ^1H n.m.r. data are reported in the experimental section.

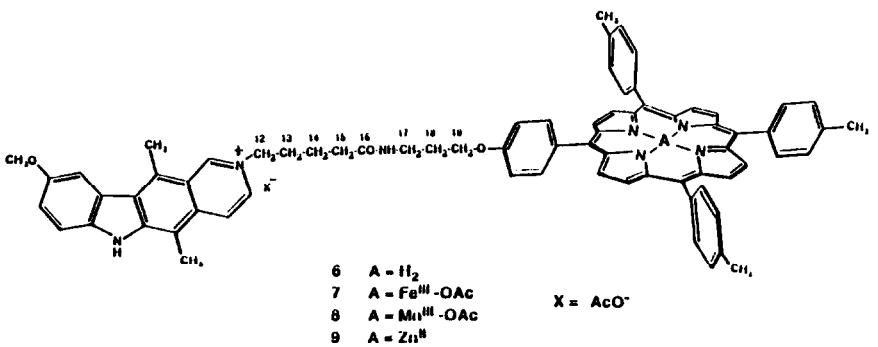


Figure 3- Structure of hybrid molecules "metalloporphyrin-ellipticine".

Because of the photosensitivity of the free base hybrid molecule, the metallation was performed directly on the crude product resulting from the coupling reaction between (3bis) and (5) in dichloromethane. The solvent is evaporated to dryness under vacuum and the residues are dissolved in dry dimethylformamide. The metallation is performed with a large excess of metal salts (10 eq) in the presence of 2,4,6-collidine at 140 °C for 2-3 hours. The completion of the reaction is followed spectrophotometrically and by analytical t.l.c. The metallated molecules are purified by dry alumina column chromatography after the exchange of counter-ions from halides to acetates (see the Experimental Section for the ion-exchange procedure).

The metallation of (6) by iron, manganese or zinc salts gave the metallated "M-porphyrin-ellipticine" molecules (7) (76%), (8) (75%), and (9) (57%), respectively (Figure 3).

The iron derivative (7) is identified by u.v.-visible data and mass spectra. A peak at 1142 has been detected in the FD-mass spectrum of (7) ($C_{73}H_{64}N_7O_3Fe,2OAc^-$) and attributed to the fragment M^+-H without two acetate ions. The immediate fragmentation of the axial ligand on the centred metal atom in metalloporphyrins is frequently encountered in mass spectrometry.[14]

Two strong bands are observed in the u.v.-visible spectra of this "metalloporphyrin- ellipticine" molecule, one at 325 nm ($\varepsilon = 39$ mmol x l^{-1} x cm^{-1}) corresponding to the ellipticine chromophore and a stronger one at 421 nm ($\varepsilon = 53$ mmol x l^{-1} x cm^{-1}) for the Soret band of the iron-porphyrin moiety.

The manganese derivative (8) ($C_{73}H_{64}N_7O_3Mn,2OAc^-$) is identified by u.v.-visible data and by the main fragment M^+-H observed at 1141 in FAB^+- or FD- mass spectra. The two strong bands for the Mn-porphyrin and ellipticine chromophores are observed at 482 ($\varepsilon = 62$ mmol x l^{-1} x cm^{-1}) and 325 (64 mmol x l^{-1} x cm^{-1}), respectively. The zinc derivative is also identified by u.v.-visible 314 nm ($\varepsilon = 93$ mmol x l^{-1} x cm^{-1}) and 420 nm (550 mmol x l^{-1} x cm^{-1}), mass spectrum and, since zinc-porphyrin complexes are diamagnetic, by ^1H n.m.r. spectroscopy. We observe two types of signals for the protons of the metalloporphyrin and the pyridocarbazole moieties. The same phenomenum is observed on the proton spectra for the free base hybrid molecule (6). The ratio of these two series of signals is not the same in (6) and (9). These features of the ^1H n.m.r. spectra for these molecules are probably due to intra- and intermolecular stacking effects and are currently under a more complete investigation (for a recent study of stacking effects and conformations in solution of flat heterodimeric molecules, see reference 15).

Nuclease Activity of Hybrid Molecules (7) and (8). Efficient oxidative cleavage of DNA was observed when the oxygen atom donor potassium hydrogen persulphate is associated with the water-soluble manganese *tetrakis*(N-methylpyridinium)porphyrin, MnTMPP.[6a] In this case the nuclease activity is observed for metalloporphyrin concentrations as low as 2.5 - 250 nM. Hemin-intercalators described by Lown *et al.* and Shudo *et al.* are less active than the manganese-tetrapyridinium derivative, their nuclease properties are obtained for concentrations ranging from 2 to 100 μM.[2b and c] The affinity of the hybrid manganese derivative (8) for Poly d(G-C) is 2 x 10^6 M^{-1}, a value similar to that ones found for hemin-intercalator (C. Auclair, personal communication).

Because the efficient binding of "metalloporphyrin-ellipticine" molecules for DNA, one would expect to obtain a high nuclease activity for these hybrid molecules. The measurements have been made on the supercoiled form of DNA from bacteriophage ΦX174 as previously described.[6a] Because their water insolubility, these two hybrid molecules (7) and (8) have been solubilised in DMF-50 mM phosphate buffer mixture (1/1, v/v). In trials the final concentration of DMF did not overcome 12.5% , conditions where DNA is not denaturated by dimethylformamide.[16] The nuclease activity of MnTMPP, used as cleaver reference, at a final concentration of 50 nM, with 10 μM potassium hydrogen

persulphate and for an incubation time of 1 minute is only reduced by a factor of five in a 12.5% DMF solution, compared to control. This small inhibition effect might result of a partial degradation of $KHSO_5$ or of the active metalloporphyrin-oxo species by DMF. In similar experimental conditions, with hybrid molecules (7) and (8), no DNA breaks are observed , even at high concentrations of hybrid molecules (250 nM, 1, 5, 10 and 20 μM) and of potassium monopersulfate (5, 10 μM, 1 and 5 mM), for incubation times of 2, 5, or 30 minutes. In order to confirm that the absence of nuclease activity is not due to a rapid chemical decomposition of (7) and (8) by the rather agressive oxygen donor, we have also studied the DNA breaks using a reducing agent like dithiothreitol, DTT, known to reduced metal complexes which are able to generate hydroxyl radicals, after reaction with dissolved molecular oxygen, and cleave DNA.[2b and 3c] In this case, with 10 mM DTT and "metalloporphyrin-ellipticine" molecules (7) or (8) at concentrations of 1, 5, 10 or 20 μM in DMF-buffer (12.5% of DMF at final concentration), even for long incubation times (1 or 20 h), no DNA cleavage are observed. In similar experimental conditions, MnTMPP (1 μM and 1 h of incubation) cleaves all DNA form I to form II (90%) and form III (10%).

The absence of nuclease activity observed for hydrid molecules (7) and (8) suggest that the high hydrophobicity of the porphyrin moiety due to the three *para*-tolyl substituents creates a repulsive effect between the cleaving part of the hybrid molecules and DNA sites.For hemin-acridines described by Lown *et al.*,[2b] efficient DNA breaks are only observed for hybrid molecules bearing a secondary amine function in the middle of the linker (at pH 7, the protonated amine forces the linker to bind to DNA, reducing then the distance between the hemin and the cleavage sites of the nucleic acid). In order to confirm this hypothesis, we are currently working on the preparation of hybrid "cationic metalloporphyrin-ellipticine" molecules which might have a much more higher DNA affinity than (7) and (8) and therefore might be able to cleave DNA with a great efficiency.

Experimental

General. 9-methoxyellipticine was a gift from the SANOFI Company (Paris). All chemicals used were of reagent grade and purchased from Aldrich. Potassium monopersulphate has been obtained from Alfa Ventron as the triple salt $2KHSO_5.KHSO_4.K_2SO_4$ (Oxone®, one mmole of monopersulphate corresponds to 307.2 mg). Dried dimethylformamide was distilled and kept over a 4 Å molecular sieve. Dried dichloromethane was distilled over calcium hydride and kept over a 4 Å molecular sieve. Column chromatography was carried out on silica gel 60 (70-230 mesh) or neutral alumina 90 (70-230 mesh) from Merck. For t.l.c. separation, Merck precoated preparative t.l.c. plates (silica gel 60, 2 mm) were used throughout this work. Ion exchange resin Amberlite IRN-78 was purchased from Prolabo. Elemental analyses were carried out by the Service de Microanalyse du Laboratoire de Chimie de Coordination (CNRS). Optical spectra in the Soret and visible region were recorded using a Varian-Cary 2300 spectrophotometer. ^1H n.m.r. spectra were obtained with a Bruker 250 WM spectrometer in the Fourier transform mode. Mass spectra were recorded using a Ribermag R1010 spectrometer for DCI (NH_3), a Varian Mat 311A spectrometer for FD. FAB^+ spectra were carried out by the Service Central d'Analyse du CNRS à Lyon.

N^2-(5-bromovaleric acid ethyl ester)-9-methoxyellipticinium bromide (2). A mixture of 9-methoxyellipticine (1) (0.102 g, 0.37 mmol) and 5-bromovaleric acid ethyl ester (0.058 ml, 0.37 mmol, 1 eq) in dimethylformamide (2 ml) was stirred at 120 °C for 4 h, then at room temperature overnight. Dry diethyl ether (20 ml) was added under stirring. The orange precipitate formed was filtered and washed with dry ether and dried in vacuum (0.156 g, 87%); λ_{max} (ε mmol x l^{-1} x cm^{-1}) in chloroform at 14 μM, 332 (6.5), 348 (3.4), 390 (4.15), 406 nm (3.9); δ_H (250 MHz; $(CD_3)_2SO$ at 303 K) 10.11 (1H, s, 1-H), 8.57 (1H, d, J = 7.1 Hz, 3-H), 8.43 (1H, d, J = 7.1 Hz, 4-H), 7.82 (1H, d, J = 1.9 Hz, 10-H), 7.62 (1H, d, J = 8.8 Hz, 7-H), 7.33 (1H, dd, J = 2.2 Hz, J = 8.7 Hz, 8-H), 4.83 (2H, t, J = 7.1 Hz, 12-H), 4.16 (2H, q, J = 7.1 Hz, 17-H), 4.02 (3H, s, OMe), 3.44 (3H, s, 11-Me), 2.84 (3H, s, 5-Me), 2.53 (2H,t , J = 7.3 Hz, 15-H), 2.16 (2H, m, 13-H), 1.73 (2H, m, 14-H), 1.28 (3H, t, J = 7.1 Hz, 18-Me).

N^2-valeric acid-9-methoxyellipticinium chloride (3). The ester (2) (0.148 g, 0.3 mmol) was refluxed under stirring in 1M hydrochloric acid (14 ml) for 3 h. The solution was allowed to stand overnight at room temperature and was then evaporated to dryness in vacuum. The residue was taken up in methanol and precipitated with ether. The orange

powder formed was filtered, washed with ether and dried in vacuum (0.125 g, 99%). The presence of chloride anions was verified by silver nitrate test. The residue was dissolved in methanol (10 ml) and was ion-exchanged on Amberlite IRN-78 in acetate form (the resin was washed three times with 10% acetic acid), after stirring at room temperature for 3 h. After filtration, the solution was concentrated in vacuum and precipitated by adding diethyl ether to the methanolic solution; ν (KBr pellets) 1656 cm^{-1} (CO); λ_{max} (ε mmol x l^{-1} x cm^{-1}) in chloroform-methanol (99:1 v/v) at 20 μM, 321 (18.0), 386 (2.5), 452 nm (1.3); δ_H (250 MHz, $(CD_3)_2SO$ at 303 K) 12.19 (1H, s, CO_2H), 10.22 (1H, s, 1-H), 8.65 (1H, d, *J* = 7.2 Hz, 3-H), 8.59 (1H, d, *J* = 7.2 Hz, 4-H), 8.05 (1H, d, *J* = 2.3 Hz, 10-H), 7.73 (1H, d, *J* = 8.7 Hz, 7-H), 7.44 (1H, dd, *J* = 8.7 Hz, *J* = 2.3 Hz, 8-H), 4.84 (2H, t, *J* = 7.0 Hz, 12-H), 4.06 (3H, s, OMe), 3.46 (3H, s, 11-Me), 3.43 (1H, s, NH), 2.97 (3H, s, 5-Me), 2.45 (2H, t, *J* = 7.4 Hz, 15-H), 2.16 (2H, m, 13-H), 1.70 (2H, m, 14-H); m/z (DCI) 377 (M^+).

[5-(4-hydroxyphenyl)-10,15,20-tris(4-tolyl)]porphyrin (4). A solution of 4-hydroxybenzaldehyde (4 g, 33 mmol, 1 eq) in propionic acid (200 ml) was warmed at 120 °C with vigorous stirring. 4-tolylaldehyde (7.7 ml, 66 mmol, 2 eq) then pyrrole (6.8 ml, 99 mmol, 3 eq) were added and the resulting mixture was refluxed for 1 h. The solution was then cooled to room temperature and the precipitate was filtered off, washed with ethanol and dried (2.01 g, 9.1%). The solid was dissolved in dichloromethane and subjected to column chromatography with dichloromethane as eluent (1.32 g, 6%); R_f (silica gel, dichloromethane-ethanol, 95:5 v/v) 0.81; Analysis (Found: C, 83.2; H, 5.5; N, 7.8. $C_{47}H_{36}N_4O$ (M = 672.79) requires C, 83.9; H, 5.4; N, 8.3%); λ_{max} (ε mmol x l^{-1} x cm^{-1}) in chloroform at 5 μM, 418 (510), 516 (18), 550 (10), 588 (5.6), 645 nm (4.8); δ_H (250 MHz, $CDCl_3$ at 295 K) 8.85 (8H, s, β-pyrrole), 8.09 (6H, d, *J* = 7.7 Hz, tolyl-2,6-H), 8.04 (2H, d, *J* = 8.35 Hz, phenolic -2,6-H), 7.54 (8H, d, *J* = 7.7 Hz, tolyl-3,5-H), 7.14 (2H, d, *J* = 8.35 Hz, phenolic-3,5-H), 2.70 (9H, s, Me), 1.55 (1H, s, OH), -2.76 (2H, s, NH-pyrrole); m/z (DCI) 673 (M^+).

{5-[4-(3-aminopropyloxy)phenyl]-10,15,20-tris(4-tolyl)}porphyrin (5). To a solution of (4) (0.150 g, 0.22 mmol) in dry dimethylformamide (10 ml) was added an excess of sodium hydroxide (crushed to a fine powder) (0.180 g, 4.5 mmol, 20 eq) and the mixture was stirred for 20 min at room temperature. 3-bromopropylamine hydrobromide (0.053 g, 0.24 mmol, 1.1 eq) was added to the green solution and the stirring was continued for 3 h at room temperature. The completion of the reaction was followed by analytical t.l.c. plates (silica gel 60, 0.25 mm). 3-bromopropylamine hydrobromide (1.1 eq) was added again and the mixture was stirred for 1 h. The solution was evaporated to dryness in vacuum and the residue was dissolved in methanol-water and extracted with dichloromethane. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was chromatographed on a silica gel column. Elution with dichloromethane-ethanol (80:20 v/v) afforded the branched porphyrin (5) as purple crystals (0.14 g, 87%); R_f (silica gel, dichloromethane-ethanol, 80:20 v/v) 0.6; λ_{max} (ε mmol x l^{-1} x cm^{-1}) in chloroform at 8.6 μM, 420 (230), 516 (8.7), 552 (5.0), 591 (2.8), 646 nm (2.3); δ_H (250 MHz, $CDCl_3$ at 295 K) 8.84 (8H, s, β-pyrrole), 8.02 (8H, s, aromatic-2,6-H), 7.43 (6H, s, tolyl-3,5-H), 7.20 (2H, s, phenoxy-3,5-H), 4.30 (2H, m, NH_2), 4.26 (2H, m, OCH_2), 3.29 (2H, m, NCH_2), 2.59 (9H, s, Me), 2.30 (2H, m, CH_2), -2.72 (2H, m, NH pyrrole); m/z (DCI) 730 (M^+).

General Procedure for the Preparation of Hybrid "Metalloporphyrin-Ellipticine" Molecules. First Step. An excess of ethyl chloroformate (0.035 ml, 0.37 mmol, 10 eq) was added dropwise to a mixture of (3) (0.070 g, 0.17 mmol, 4.5 eq) and triethylamine (0.037 ml, 0.26 mmol, 7 eq) in dry dichloromethane (3 ml). The mixture was stirred at room temperature for 0.5 h and evaporated to dryness under vacuum. The residue was dissolved in dry dichloromethane (3 ml). Triethylamine (0.037 ml, 0.26 mmol, 7 eq) then (5) (0.028 g, 0.038 mmol) were added. The mixture was heated under reflux for 2 h then cooled to room temperature. The solvent was evaporated to dryness under vacuum and the residue was dissolved in dichloromethane. The resulting solution was submitted to preparative silica gel t.l.c. and developed with dichloromethane-ethanol (80:20 v/v) in the dark. ((6), 0.020 g, 47%); R_f (silica gel, dichloromethane-ethanol, 80:20 v/v) 0.66; λ_{max} (ε mmol x l^{-1} x cm^{-1}) in chloroform 320 (27), 420 (110), 518 (4.9), 548 (2.5), 586 (1.5), 644 nm (2.0); δ_H (250 MHz, $(CD_3)_2SO$ at 295 K) 10.24 (45%) and 10.21 (55%) (1H, 2 x s, 1-H), 8.93 and 8.92 (8H, 2 x s, β-pyrrole), 8.59 (2H, m, 3-H and 4-H), 8.20 (6H, d, *J* = 7.8 Hz, tolyl-2,6-H), 8.15 (2H, d, *J* = 8.7 Hz, phenolic-2,6-H), 8.02 (1H, d, *J* = 2.3 Hz, 10-H), 7.89 (1H, d, *J* = 2.3 Hz, 10'-H), 7.74 (6H, d, *J* = 7.7 Hz, tolyl-3,5-H), 7.59 (1H, d, *J* = 8.8 Hz, 7-H), 7.42 (2H, 2 x d, *J* = 8.7 Hz, phenoxy-3,5-H), 7.28 (1H, dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 8-H), 4.83 (2H, m, 12-H), 4.37 (2H, m, 19-H), 4.05 (55%) and 3.94 (45%) (3H, 2 x s, OMe), 3.69 (3H, s, 11-Me), 2.96 (55%) and 2.86 (45%) (3H, 2 x s, 5-Me), 2.78 (9H, s, Me), 2.53 (2H, m, 15-H), 2.39 (2H, m, 15'-H), 2.10 (4H, m, 13-H and 18-H), 1.72 (4H, m, 14-H and 17-H), -2.86 (2H, s, NH pyrrole); m/z (FD) 1088 (M^+-H); m/z (FAB$^+$) 1088 (M^+-H).

Second Step. The metallation of the hybrid molecule (6) can take place without purification of the later. So, after evaporation of dichloromethane, the residue was dissolved in dry dimethylformamide (3 ml). 2,4,6-collidine (0.05 ml, 0.38 mmol, 10 eq) was added and the solution was warmed to 100 °C. An excess of manganese acetate tetrahydrate, iron (II) chloride tetrahydrate or zinc acetate dihydrate (10 eq) was then added and the mixture was heated at 140 °C for 2-3 h. The completion of the reaction was followed spectrophotometrically and by analytical t.l.c. The reaction mixture

was then cooled to room temperature. Water (15 ml) was added and the mixture was stirred at room temperature for 1 h. The precipitate was filtered and washed with water (3 x 10 ml). The solid was dissolved in methanol and was ion-exchanged on Amberlite IRN-78 in acetate form after stirring at room temperature for 3 hours.

After filtration, the solution was evaporated to dryness and chromatographed on dry alumina column (elution with dichloromethane-ethanol (80:20 v/v) for Fe(III) and Mn(III) porphyrins (**7**, **8**) and dichloromethane-ethanol (96:4 v/v) for zinc porphyrin (**9**).

(**7**) (0.037 g, 76%); R_f (silica gel, dichloromethane-ethanol, 80:20 v/v) 0.27; λ_{max} (ε mmol x l^{-1} x cm^{-1}) in chloroform at 15.5 µM, 325 (39), 421 nm (53); m/z (FD) 1142 (M^+-H).

(**8**) (0.036 g, 75%); R_f (silica gel, dichloromethane-ethanol, 80:20 v/v) 0.33; λ_{max} (ε mmol x l^{-1} x cm^{-1}) in chloroform at 5.9 µM, 325 (64), 386 (44), 482 (62), 582 (7.6), 620 nm (9.6); m/z (FD) 1141 (M^+-H); m/z (FAB^+) 1141 (M^+-H).

(**9**) (0.026 g, 57%); R_f (silica gel, dichloromethane-ethanol, 80:20 v/v) 0.18; λ_{max} (ε mmol x l^{-1} x cm^{-1}) in methanol at 6.7 µM, 314 (93), 420 (550), 555 (21), 596 nm (10); δ_H (250 MHz, $(CD_3)_2SO$ at 296 K) 10.26 (85%) and 10.22 (15%) (1H, 2 x s, 1-H), 8.88 (8H, s, β-pyrrole), 8.66 (1H, d, J = 7.2 Hz, 3-H), 8.58 (1H, d, J = 7.2 Hz, 4-H), 8.16 (8H, d, J = 7.7 Hz, aromatic-2,6-H), 8.04 (1H, d, J = 2.1 Hz, 10-H), 7.69 (7H, m, p-tolyl-3,5-H and 7-H), 7.39 (3H, m, phenoxy-3,5-H and 8-H), 4.86 (2H, t, J = 7.5 Hz, 12-H), 4.36 (2H, t, J = 6.25 Hz, 19-H), 4.06 (15%) and 4.01 (85%) (3H, 2 x s, OMe), 3.44 (3H, s, 11-Me), 2.97 (15%) and 2.92 (85%) (3H, 2 x s, 5-Me), 2.78 (9H, s, Me-p-tolyl), 2.38 (2H, t, J = 7.5 Hz, 15-H), 2.11 (4H, m, 13- and 18-H), 1.77 (4H, m, 14- and 17-H); m/z (FAB^+) 1151 (M^+-H).

Experimental conditions for the DNA breaks study. The DNA cleavage studies were performed on supercoiled ΦX174 DNA (from Bethesda Research Laboratories, purity: 90%) in the following conditions:

ΦX174 DNA digestion conditions. For all the experiments, DNA was diluted in phosphate buffer (5mM, pH 7.4). Each digestion reaction was performed on a total volume of 20 µl containing 5 µl of ΦX174 DNA (3.5 nM, base pair concentration: 18.8 µM), 5 µl of a metalloporphyrin solution in DMF-50 mM phosphate buffer pH 7.4 (1/1,v/v) (final concentrations: 250 nM, 1, 5, 10 and 20 µM), 5 µl of 50 mM phosphate buffer pH 7.4 and 5 µl of potassium monopersulphate diluted in the same buffer (final concentrations: 5 and 10 µM, 1 and 5 mM) or 5 µl of 10 mM (final concentration) DTT in the same buffer. The final concentration of DMF was never allowed to exceed 12.5%, even though DMF is a poor denaturant for DNA.[16] The reactions were allowed to proceed at 20°C for 2, 5, or 30 min in the presence of potassium persulphate (or for 1 or 20 h in the presence of DTT). DNA and the metalloporphyrin derivative were pre-incubated for 20 min.

Electrophoresis conditions. Metalloporphyrin-mediated DNA cleavage was monitored by agarose gel electrophoresis. After incubation, reactions were quenched by 5 µl of a "stopping reagent" and samples were kept on ice. The stopping reagent consisted of 250 mM HEPES pH 7.2 buffer containing 75% of glycerol and 0.05% of bromphenol blue. We checked that 50 mM HEPES buffer, pH 7.2 (final concentration in quenched reaction samples) degrades more than 90% of potassium persuphate within one minute. Control experiments showed no DNA strand scission by the degradation products.

Electrophoreses of treated reaction mixtures were then run in 0.8% agarose slab horizontal gel, containing 1 µg per ml of ethidium br de, at constant current (25 mA for 16 h), in 89 mM Tris-borate buffer, pH 8.3. Bands were located by u.v. ligh photographed.

Quantification of t nount of DNA forms (I,II or III) was done by densitometry (Hoefer GS-300). The observed fluorescence of form I was corrected (correction factor: 1.47, standard error: 0.3).

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