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Oxidative Cleavage of Plasmid Bluescript by Water-Soluble Mn-Porphyrins and Artificial Oxidants or Molecular Oxygen

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Abstract—A set of eight Mn(III)-porphyrins were used as catalysts in oxidative demolition of Plasmid Bluescript, to the nicked and linear forms, in the presence of different oxygen donors (NaOCl, H_2O_2, AcOOH, *t*-BuOOH). The efficiency of the catalytic system is related to a combination of factors such as porphyrin structures, pH of the aqueous phase and nature of the primary oxidant. The highest catalytic activity was observed when ionic porphyrins were used as catalyst (the cationic being more active than the anionic) and NaOCl was used as primary oxidant at pH 9.5; in contrast, lipophilic catalysts proved to be completely unreactive towards the DNA, whichever oxidant used. The plasmid demolition was also achieved by irradiating the reaction mixture, containing Zinc porphyrins, with a white lamp; under these conditions, the highest efficiency was again observed with *meso*-tetra(1-methyl-4-pyridyl)porphyrin. However, preliminary experiments of photo activation applied on tumour cells (HCT 116) showed no dead cells with cationic porphyrin, while the amphiphilic Zn-tetra(4-hydroxyphenyl)porphyrin gave IC_{50} values at 5×10^{-2} μM concentration (37.1 ng/mL).

Introduction

Oxidative properties of metallo-tetraarylporphyrins towards alkanes, alkenes and other substrates in the presence of a single oxygen atom donor (OD) such as sodium hypochlorite (NaOCl), hydrogenperoxide (H_2O_2), alkyl hydroperoxydes (ROOH) and peracids (RCOOH) are well established and deeply investigated.[1–5]

The mechanism of oxygen transfer from the OD to the substrate provides for the formation of a high-valent metallo-oxo species, whose characteristics depend on the nature of the metal,[6–8] then, as final step of the reaction pathway, the oxygen atom is transferred to the substrate with the consequent formation of alcohols and epoxides from saturated and unsaturated hydrocarbons, respectively. With transition metallo-complexes, the reactivity of the metallo-oxo species depends on the oxidation state of the metal, as particularly evidenced in the case of Mn(III)-porphyrins which can generate either Mn(IV) or Mn(V)-oxo species.[9] It has to be pointed out that the mechanism of metallo-oxo species formation also depends on the type of the OD used as primary oxidant and, furthermore, the kinetic of formation is particularly affected by the pH values of the aqueous phase.[10–13]

Two further remarkable features of porphyrins, in particular of the hematoporphyrin derivatives (HPD), are the preferential absorption in neoplastic tissues and the interaction with nucleic acids;[14–18] this last property, found in both free-base or metallo-tetrapyrrole macrorings, has been widely studied since the beginning of the 1980s.[19] The interpretation of analytical data [linear dichroism (LD) or circular dichroism (CD),[20–23] Dnase I footprinting methodology,[24] apparent affinity binding constants evaluation[25] and the changing of the values of viscosity or of melting point[26] of DNA], mainly obtained with studies on calf thymus DNA or synthetic homo-nucleotide polymers, brought to the identification of several ways of interaction between DNA and porphyrins. According to these studies, two most important modes of interaction were recognized: (1) *intercalation at GC reach regions*, evidenced by hypochromicities of the Soret band, negative CD signals, relatively high binding constant and by both viscosity and melting point increases; (2) *outside self-stacking at the AT regions*, characterized by minor changes of the UV–visible spectra, positive CD signals, slight decrease of the viscosity, and increase of melting point of 1–2 °C.

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As a general rule, tetraarylporphyrins lacking bulky groups on the *meso* phenyls or coordinated on the central metal (i.e., absence of hindrance out of the porphyrin plane) give intercalation, independently of the number of charges present on the skeleton; on the other hand, porphyrins with large substituents, pointing out of the tetrapyrrole ring plane, give external binding.

Independently of the mode of binding, the ability of porphyrin to penetrate cell membranes, targeting different cell's organism, together with porphyrin light absorption and emission properties, are the characteristics that, since the beginning of the 1980s, led the investigation to the application of this class of molecules in cancer therapy.[22,24]

The evolution of the research in this field has brought to the development of porphyrins and related compounds, (chlorins, bacteriochlorins) and other molecules (phthalocyanines, verteporfin, texaphyrins) as photosensitizers in tumour photodynamic therapy (PDT). Since tissues are deeper penetrated by a 700–800-nm electromagnetic wave, the common feature of photosensitizers should be an intense absorbance in the red field of the visible spectrum.

The interest in PDT belong on its fundamental characteristics:

1. It is a not invasive therapy which finds particular interest for those tumours that can be easily reached with a light of suited features.
2. Can be associated to other therapies such as chemotherapy or radiotherapy.

The irradiating light should be characterized by a wavelength of emission in the red spectral region, with a power density strong enough (75–150 mW/cm^2) to reach a considerable energy density (130–150 J/cm^2) in a short irradiation time.[27–29]

The oxidizing property of the porphyrin/light system is imputed to the formation of the high energy singlet oxygen from the triplet state molecular oxygen; the process relies on light absorption by the macrocycle (a fully conjugated unsaturated system which acts as chromophore) followed by energy transfer to the O_2 present in the medium. The singlet oxygen thus generated, a strong oxidant behaving as radical, reacts with any of the organic molecules within a 100 Å distance. Its mechanism of action is comparable to the formation of radical species (HO°, HOO°) in the above mentioned metallo-porphyrin/OD system, which accounts for the formation of alcohols from saturated hydrocarbons;[30] indeed the generation of hydroxy or hydroperoxy radicals can not be excluded in the photo-catalyzed processes too.[16]

On the basis of these observations we decided to investigate the effect of a catalytic system (manganese-tetraarylporphyrin/oxygen donor) on the oxidative demolition of supercoiled plasmid Bluescript to the corresponding nicked or linear forms. In detail, we here report the effect of several oxygen donors, sodium hypochlorite (NaOCl), hydrogenperoxide (H_2O_2), *tert*-butylhydroperoxide (*t*-BuOOH) and peracetic acid (AcOOH), with regard to:

i. oxidant concentrations and pH of reaction medium;
ii. manganese-porphyrin concentrations ([Mn-P]) and ratio with respect to the plasmid;
iii. structural characteristics of porphyrin skeleton over a series of eight different compounds (featuring positive, negative or amphiphilic moieties).

Furthermore, two Zn-complexes, of either positively charged or neutral porphyrins, were used to induce the photo-catalyzed cleavage of plasmid Bluescript by irradiating with a slide projector fitted with a 150-W halogen lamp. An ancillary experiment on porphyrin photoactivations was also carried out on human colon tumour cell (HCT 116).

Results and Discussion

It is well known that artificial oxidant aqueous solutions are stable at an appropriate pH, however, when associated with catalytic amount of metallo-porphyrins, each OD shows the best catalytic efficiency under modified pH conditions. In particular, sodium hypochlorite aqueous solution is stable under strong basic conditions (pH 13–14) however it was successfully used in hydrocarbon oxygenations at pH 9.5;[31] 30% hydrogen peroxide is stable at low pH values (pH 2–3), nevertheless was conveniently used at pH 4.5–5.0 in alkane and alkene mono-oxygenations catalysed by Mn-porphyrins. In this case, the pH of the H_2O_2 solution was increased by the addition of carboxylic acid salts; these last were found to behave either as buffer as well as co-catalysts.[32] Among the oxidants investigated in this work, only *t*-BuOOH is available as neutral aqueous solution while peracetic acid is commercially available in acid medium (39% solution in acetic acid).

Aware of the dependence of the reactivity of Mn-porphyrin/OD catalytic system on the pH values, we have initially investigated the range of pH under which the plasmid Bluescript maintains the supercoiled structure for at least 1 h at 25 °C. It was found that the plasmid was stable in buffered aqueous solutions in the 5.0–10.7 pH range; indeed none of the nicked or linear forms were observed after 1 h at room temperature, as determined by fluorescence after electrophoresis on agarose gel.

Taking into account this result we have then investigated the effect of each OD in relation to a particular pH, in order to determine the maximum OD concentration ($[OD]_{max}$) which does not produce oxidative damage to the plasmid ([Plasmid] = 10^{-7} M, i.e., 3×10^{-4}

M in base pairs] within 30 min of reaction. The results, reported in Table 1, for NaOCl, H_2O_2, AcOOH and *t*-BuOOH, respectively, indicate the following OD/DNA^{bp} ratio and pH: NaOCl/DNA^{bp} = 1 (pH 9.2); H_2O_2/DNA^{bp} = 10 (pH 5.0); AcOOH/DNA^{bp} = 1 (pH 9.2); *t*-BuOOH/DNA^{bp} = 100 (pH 9.2).

The correlation between pH and OD activity deserves a comment: about NaOCl it is known that lowering the pH of the aqueous solution from pH 13 up to pH 9.5, causes the formation of hypochlorous acid (HClO) which is even more reactive than NaOCl.[31] A further decrease of pH generates chlorine which is a particularly reactive molecule which reacts also in the absence of metallo-catalysts. In the case of hydrogen peroxide, by increasing the pH of the aqueous solution another oxidizing specie can be generated; at pH 8.0–9.0 a nucleophilic, powerful oxidant (HOO^-) becomes the active species. Under higher basic conditions, a fast H_2O_2 decomposition to O_2 and H_2O is observed. In alkene epoxidations, catalyzed by Mn-porphyrins, H_2O_2 was conveniently used at pH 4.5–5.0,[32,33] accordingly, we decided to test this oxidant under those pH conditions. In the case of both AcOOH and *t*-BuOOH, the activity seems to be independent of the pH of the plasmid buffered solution, nor peculiar reactivity of these two oxidants is known in the pH range here investigated; hence these oxidants were used at pH 9.2 for a straight comparison of the results with those obtained with NaOCl.

As far as the reactivity is concerned, the OD/DNA ratios indicate that the alkylhydroperoxide has, by far, a lower activity compared to that one of the other oxidants, while peracetic acid has a comparable activity with respect to NaOCl. Hydrogen-peroxide behaves in a mean way; indeed a partial demolition was observed when an excess of 10 times of H_2O_2 was used with respect to the plasmid, compared to the 1/1 ratio used for NaOCl and AcOOH and to the 100/1 ratio for *t*-BuOOH.

The following step of the research was the determination of the maximum Mn-porphyrin concentration ($[Mn\text{-porphyrin}]_{max}$), which, individually, does not induce plasmid partial cleavage. For this purpose, we used the manganese complex of *meso*-tetra(4-methylpyridinium)porphyrin [Mn-T(4PyMe$^+$)P] **Mn-1**, which is easily obtained by inserting manganese into the commercially available free base methylpyridinium-porphyrin (**H_2-9**). Furthermore this porphyrin was chosen because it has been, and it is still now, the most frequently used porphyrin in DNA intercalation studies.[34,35] The Mn-complexes were chosen because, among the metallo-tetraarylporphyrins (metal = Mn, Fe, Co, Ru, Ti, etc.) which are known to be active in catalytic oxidations, Mn-porphyrins proved to be the most efficient catalysts (i.e., number of moles of substrate oxidised per mole of catalyst) according to the results obtained by us in the catalytic oxidation of saturated and unsaturated hydrocarbons.[36,37]

The results (Table 2) indicate that up to a ratio Mn(P)/DNA^{bp} = 0.01 **Mn-1** is ineffective on plasmid degradation for at least 30 min at pH 9.2 and 25 °C.

On the basis of the results reported above, the conditions (i.e., the initial catalyst concentration, oxidant concentrations and pH values) suitable for the study of the catalytic efficiency of differently structured metallo-tetraarylporphyrins, have been correctly defined. The complete catalytic system, consisting of Mn(P) and OD, was then evaluated on the oxidative demolition of plasmid Bluescript; the investigation dealt with a model system composed by the Mn-T(4PyMe$^+$)P (**Mn-1**) and NaOCl, afterwards the activity of this catalyst was studied in presence of the other three oxidants; in the last part of the work, the activities of differently structured Mn-catalysts were evaluated in the presence of NaOCl.

Table 3 shows the results of plasmid oxidation catalysed by **Mn-1** in the presence of the above-mentioned ODs.

In the presence of NaOCl, at pH 9.2, with a [DNA^{bp}] = 3.0×10^{-4} M (which means a $[plasmid]_{initial}$ = 0.1 μM) and DNA^{bp}/OD/**Mn-1** = 1/1/0.001 ratio (i.e., three

Table 1. Plasmid oxidative damage: pH and concentration effect

	pH	Concn (M)	Supercoiled			Nicked			Linear		
			5′	20′	30′	5′	20′	30′	5′	20′	30′
NaOCl	9.2	3×10^{-3}	0	0	0	0	0	0	100	0	0
	9.2	3×10^{-4}	100	100	100	0	0	0	0	0	0
	10.7	3×10^{-4}	100	100	100	0	0	0	0	0	0
H_2O_2	9.2	3×10^{-3}	80	60	10	20	40	80	0	0	10
	5	3×10^{-3}	100	100	100	0	0	0	0	0	0
	5	3×10^{-4}	100	100	100	0	0	0	0	0	0
AcOOH	9.2	3×10^{-4}	100	100	100	0	0	0	0	0	0
	10.7	3×10^{-4}	100	100	100	0	0	0	0	0	0
	5	3×10^{-4}	100	100	100	0	0	0	0	0	0
tBuOOH	9.2	3×10^{-2}	100	100	100	0	0	0	0	0	0
	10.7	3×10^{-2}	100	100	100	0	0	0	0	0	0
	5	3×10^{-2}	100	100	100	0	0	0	0	0	0

[DNA] = 3×10^{-4} M (bp).

Table 2. Effect of [Mn−T(4PyMe$^+$)P] in plasmid damage

Mn-1 (M)	pH	Supercoiled			Nicked			Linear		
		5′	20′	30′	5′	20′	30′	5′	20′	30′
3×10^{-7}	9.2	100	100	100	0	0	0	0	0	0
3×10^{-6}	9.2	100	100	100	0	0	0	0	0	0
3×10^{-5}	9.2	100	80	60	0	20	40	0	0	0

[DNA] = 3×10^{-4} M (bp).

Table 3. Plasmid oxidative damage with **Mn-1** and four ODs

OD	[OD] (M)	[**Mn-1**] (M)	pH	Supercoiled			Nicked			Linear		
				5′	10′	20′	5′	10′	20′	5′	10′	20′
NaOCl	3×10^{-4}	3×10^{-7}	9.2	0	0	0	80	60	40	20	40	60
AcOOH	3×10^{-4}	3×10^{-7}	9.2	0	0	0	90	70	50	10	30	50
H_2O_2	3×10^{-4}	3×10^{-6}	5	0	0	0	60	60	60	40	40	40
tBuOOH	3×10^{-2}	3×10^{-6}	9.2	0	0	0	50	50	50	50	50	50

[DNA] = 3×10^{-4} M (bp).

molecules of catalyst per DNA), the supercoiled DNA was degraded to the nicked and linear forms within 5 min; the progression of the oxidative demolition with the time was confirmed by the increase of the amount of the linear species with respect to the nicked one, the latter diminishes from 60 to 40% in the last 10 min of reaction (see Fig. 1 for an example of electrophoresis gel).

Comparable results were obtained with peracetic acid under equivalent pH conditions; the supercoiled plasmid was cleaved into the nicked and linear species with a comparable rate, the two forms being 50% each after 20 min.

A different behaviour was instead observed in the case of hydrogen peroxide: under the conditions used in the presence of NaOCl and AcOOH (DNA^{bp}/OD/**Mn-1** = 1/1/0.001) no plasmid demolition was observed; this result was not unexpected taking into account that H_2O_2 alone requires a 10 times excess with respect to the plasmid to induce demolition. We decide to verify if the plasmid damage could be produced either increasing the oxidant or catalyst concentration; it was found that increasing of 10 times the amount of catalyst **Mn-1**, both linear and nicked forms were generated although keeping constant the DNA^{bp}/H_2O_2 ratio. Under these conditions the demolition of the supercoiled form was exhaustive within the first 5 min and the linear DNA was detected in 40% yield; this degree of demolition is twice than the one obtained in the presence of NaOCl and 4 times the one obtained with AcOOH at the same reaction time. However, the ratio between the linear and nicked forms did not change with the reaction time, thus indicating the loss of efficiency of the catalytic system.

As reported above, among the oxygen donors considered in this work, *t*-BuOOH is the less reactive, the presence of metal catalysts being always necessary to activate this oxidant.[13,38] Its low activity has been confirmed on both Mn-porphyrins alkene and DNA catalytic oxidations; indeed the plasmid oxidative demolition was observed in good extent only when catalyst and *t*-BuOOH concentrations were increased of 10 and 100 times, respectively. As already found with H_2O_2, also in the presence of *t*-BuOOH, the plasmid cleavage suddenly ceased after the first 5 min; the amount of nicked and linear forms actually remains constant in the following 15 min, thus indicating the deactivation of the catalytic system after few min. This observation needs a comment.

It is known that metallo-porphyrins undergo a partial demolition (bleaching) when used as catalyst in hydrocarbon oxygenations. Many efforts have been done to understand the factors influencing the stability of the tetrapyrrole-macroring, in the presence of ODs; finally, the characteristics of both the nature and the position of the peripheral substituents which provide stability to the tetraphenyl-porphyrin skeleton, were established.[39,40] The presence of bulky and electron withdrawing groups on the *ortho, ortho′* positions of *meso* phenyl rings was found crucial to achieve the required stability.

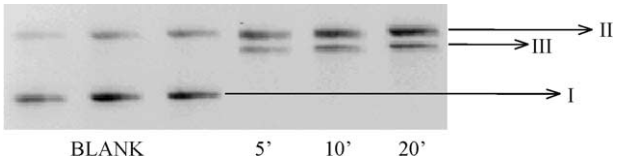


Figure 1. Agarose gel showing cleavage of Plasmid Bluescript catalyzed by **Mn-1** in the presence of NaOCl, at pH 9.2 and 25 °C. The symbols I, II, and III refer to closed circular, nicked circular, and linear DNA, respectively.

Catalytic activity of Mn-complexes [Mn-(2–8)]

The research on catalytic activities of differently structured porphyrins, featuring positively charged moieties, negatively charged groups or amphiphilic substituents, was then addressed. The catalyst structures are reported in Figure 2.

In the case of porphyrins **Mn-2**, another catalyst widely used in the evaluation of porphyrins/DNA interaction,[34] the manganese complex was easily prepared by metallation, under standard conditions, of the commercially available free base porphyrin (**H_2-10**). The catalytic activity of **Mn-2** was assessed in the presence of three out of the four ODs (NaOCl, AcOOH and H_2O_2); the exclusion of *t*-BuOOH was dictated by its poor activity compared with that one of the other oxidants. The results are reported in Table 4.

With this catalyst, a prevalent amount of the starting supercoiled plasmid is always present in the reaction mixture and the formation of the linear form was never observed; the nicked species reaches a maximum of 30%

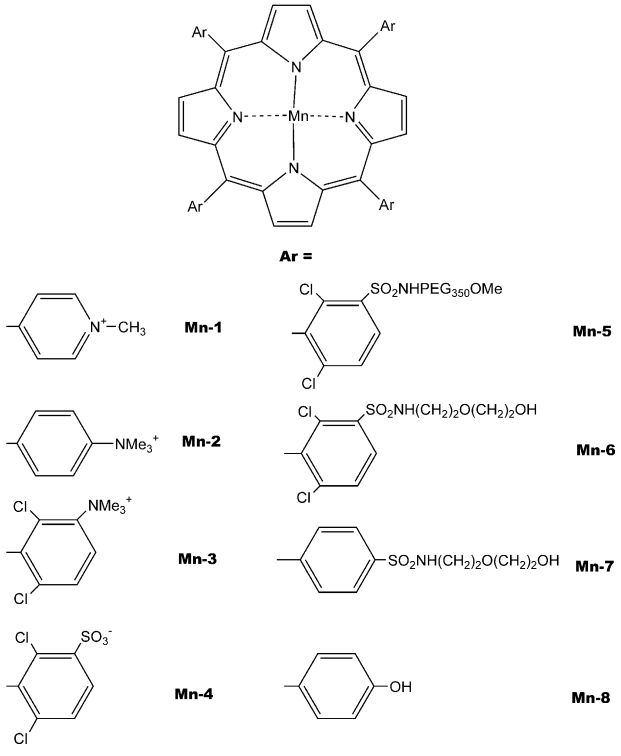


Figure 2. Structures of tetraarylporphyrins manganese chloride complexes used as catalysts in the oxidative demolition of Plasmid Bluescript.

Table 4. Plasmid oxidative damage with **Mn-2** and three ODs

OD	[OD] (M)	[**Mn-2**] (M)	pH	Supercoiled			Nicked			Linear		
				5′	20′	40′	5′	20′	40′	5′	20′	40′
NaOCl	3×10^{-4}	3×10^{-7}	9.2	90	80	70	10	20	30	0	0	0
H_2O_2	3×10^{-4}	3×10^{-6}	5	100	80	70	0	20	30	0	0	0
AcOOH	3×10^{-4}	3×10^{-7}	9.2	50	30	0	50	70	90	0	0	10

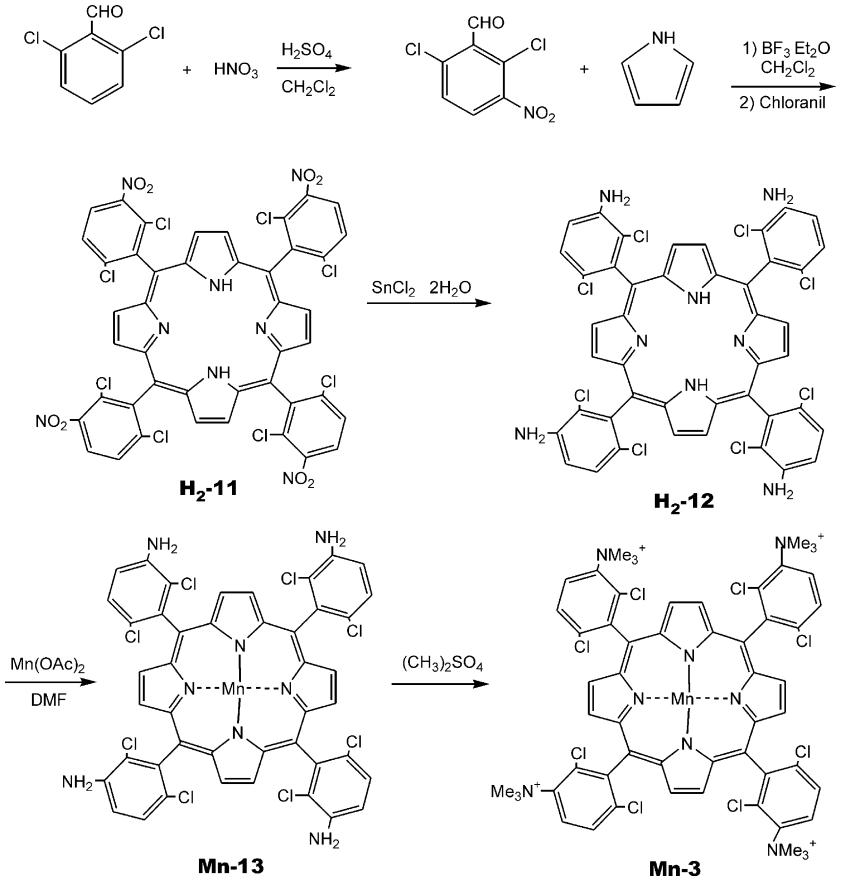
[DNA] = 3×10^{-4} M (bp).

with sodium hypochlorite (or with H_2O_2) only after longer reaction time (40 min) with respect to those found with catalyst **Mn-1**. In the presence of peracetic acid the activity of **Mn-2** was even lower.

The different behaviour of catalyst **Mn-2** with respect to **Mn-1** could be ascribed to the structure of their skeletons, which differ in: (a) electronic effects or (b) steric effects. The former are ruled by the position of the positive charges, which assert a different electronic distribution on the tetrapyrrole macroring; in compound **1** the positive charges are on the phenyls ring (pyridinium salts) while in compound **2** are on the adjacent nitrogen atom (anilinium salts). The different electronic effects of these groups could influence the electronic density on the metal, afterwards on both the formation and the reactivity of the manganese-oxo species. However several studies indicate that the electronic effects have poor influence on the kinetics of the reaction between Mn- or Fe-porphyrins and OD.[12,37,38] The steric effect seems to be more important; according to Marzilli,[26] the bulkiness of tetramethylammonium group (about 6 Å thick) can be sufficient to inhibit the intercalation on CT DNA; with this DNA the intercalation, which occurs in GC-rich regions, is assured by the presence of methylpyridiniun groups.

On the basis of these observations a new question arise: is intercalation strictly necessary to obtain an efficient oxidative process on a supercoiled plasmid?

In order to stress the effect ascribable to steric hindrance, we synthesized a new 'sterically demanding' porphyrin characterized by the presence, on the *ortho*, *ortho′* positions each phenyls, of two electron withdrawing and bulky substituents (chlorine atoms) and one trimethylammonium group in *meta* position (**Mn-3**) The synthesis of this new compound was achieved in four steps starting from the 2,6-dichlorobenzaldehyde which was nitrated on position 3, then condensed with pyrrole in order to get the corresponding porphyrin [*meso*-tetra(2,6-dichloro-3- nitrophenyl)porphyrin, **H_2-11**]. This porphyrin was first reduced to the tetra-amino derivative (**H_2-12**) with $SnCl_2$ in refluxing ethyl acetate, afterwards converted into the manganese complex (**Mn-13**) refluxing the porphyrin DMF solution in the presence of large excess of manganese salts. The four amino groups were then exhaustively methylated with dimethylsulphate to produce the corresponding trimethylammonium ions (**Mn-3**) (Scheme 1).



Scheme 1. Synthesis of catalyst **Mn-3**.

Since the results on plasmid Bluescript demolition with catalyst **Mn-2** were found dependent on the oxidant used, we chose to investigate the activity of the other catalysts only in the presence of NaOCl.

Quite surprisingly, despite the higher hindrance due to the presence of chlorine atoms on the *ortho, ortho'* positions (which inhibit the free rotation of the '*meso*' phenyls, therefore preventing the achievement of a planar conformation with respect to the tetrapyrrole ring), catalyst **Mn-3** gave the same results of **Mn-2**, thus indicating that a severe steric hindrance has no or little effect on catalyst activities (Table 5). Therefore, the different activity between catalysts **Mn-2** (or **Mn-3**) and **Mn-1** should find an explanation only considering the two different ways of interaction with DNA suggested (i.e., pyridinium porphyrin intercalates while the anylinium porphyrin does not). However, while a huge amount of information is available on linear DNA, no data are available on the metallo-porphyrins interaction with supercoiled DNA. Recently Tabata reports a study one Bluescript plasmid concerning the effect of a type II restriction enzyme (HaeIII) in the presence of Hg-, Cd-, Pb-methylpyridinium porphyrins, however, no spectral data are available on direct interaction.[41] Hence, we decided to evaluate the UV–vis spectral changes of porphyrin Soret band in the presence of the plasmid Bluescript. The titrations were carried out either by the additions of a concentrate solution of **Mn-1** ($1–0.05\times10^{-5}$ M) to a standard DNA (50 μg/mL) solution or vice versa; in both cases, no shift of the porphyrin Soret band could be observed nor iperchromic or ipochromic effects, thus indicating that **Mn-1** does not intercalate with plasmid Bluescript.

To investigate deeper how much the catalyst structures could influence the Mn-porphyrin catalytic activity on Bluescript plasmid, we synthesized the negatively charged **Mn-4** which features analogous steric hindrance than porphyrin **Mn-3**, however with negatively charged groups (sulphate anions) in place of positively charged trimethylammonium moieties. **Mn-4** was obtained by direct chlorosulphonation of the 2,6-dichlorophenylporphyrin (**H_2-14**) with chlorosulphonic acid at 100 °C,[42] to give **H_2-15** porphyrin, followed by the hydrolysis of the tetra-sulphonylchloride intermediate with stoichiometric amount of K_2CO_3 affording the tetra-potassiumsulphonate derivative **H_2-16** (Scheme 2). The manganese insertion was obtained refluxing a porphyrin water solution with an excess of $Mn(OAc)_2$.

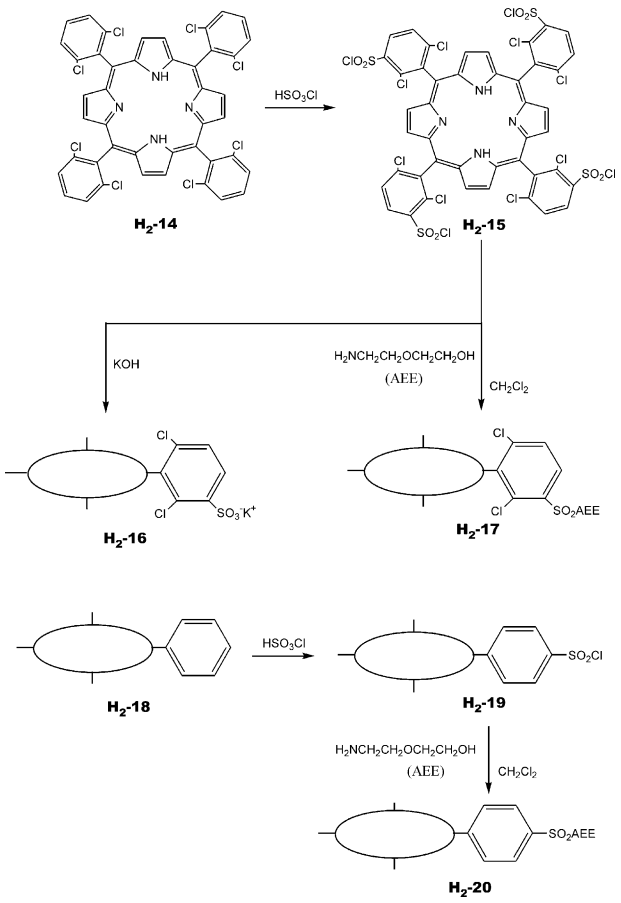
Table 5. Plasmid oxidative damage with NaOCl and different catalysts **Mn-(3–8)**

Mn	[Mn] (M)	pH	Supercoiled			Nicked			Linear		
			5′	20′	40′	5′	20′	40′	5′	20′	40′
3	3×10^{-7}	9.2	95	80	70	5	20	30	0	0	0
4	3×10^{-7}	9.2	95	80	20	5	10	20	0	0	0
5	3×10^{-7}	9.2	100	100	100	0	0	0	0	0	0
6	3×10^{-7}	9.2	100	100	100	0	0	0	0	0	0
7	3×10^{-7}	9.2	100	100	100	0	0	0	0	0	0
8	3×10^{-7}	9.2	100	100	100	0	0	0	0	0	0

[DNA] = 3×10^{-4} M (bp), [NaOCl] = 3×10^{-4} M.

Although **Mn-4** catalytic activity was studied with three oxidants (NaOCl, H_2O_2, AcOOH), it showed DNA partial demolition only in the presence of NaOCl, and with a lower efficiency with respect to the above mentioned positively charged catalysts, **Mn-3** (Table 5). Indeed it is well known that the interaction of negatively charged molecules with DNA is particularly weak because of the electrostatic repulsion between the four sulphate anions of the catalyst and the phosphate groups of the DNA.

The investigation of catalysts reactivity/structure relationship proceeded with the analysis of catalysts featuring non ionic substituents on the phenyl rings [**Mn-(5–8)**]. The porphyrin skeletons were functionalized with four polar tails; the desired effect was to infer amphiphilic character to the molecules. Two catalysts (**Mn-5**, **Mn-6**) were synthesized by reaction of hydrophilic amines on the *meso*-tetra(2,6-dichloro-3-sulphonylchloride-phenyl)porphyrin (**H_2-15**), followed by manganese insertion. The synthesis of catalyst **Mn-5** was previously described,[43] while **Mn-6** was synthesized from **H_2-15** by reaction with the commercially available 2-(2-aminoethoxy)ethanol yielding the free base porphyrin **H_2-17** (Scheme 2), from which **Mn-6** was obtained. Catalyst **Mn-7**, characterized by the presence of the corresponding sulphonamido lateral chains as in



Scheme 2. Synthesis of sulphonated free base porphyrins **H_2-16**, **H_2-17**, and **H_2-20**, precursors of manganese-porphyrins **Mn-4**, **Mn-6**, and **Mn-7**, respectively.

compound **Mn-6**, was synthesized by chlorosulphonylation of H_2-tetraphenylporphyrin (**H_2-18**), affording the porphyrin **H_2-19**, followed by the reaction with 2-(2-aminoethoxy)ethanol (Scheme 2). The product (**H_2-20**) was then metallated under standard conditions.

The last catalyst considered was the manganese complex (**Mn-8**) of the commercially available H_2-tetra(4-hydroxyphenyl)porphyrin, (**H_2-21**). This molecule was chosen because it features intermediated characteristics of polarity and bulkiness with respect to the above mentioned catalysts, actually **Mn-8** brings only four small polar groups (–OH) on the *para* position of each phenyls.

The activity of catalysts **Mn-(5–8)** was studied in the presence of NaOCl. Unfortunately none of these catalysts proved to be active on plasmid demolition, the nicked form being seldom detectable as tiny spots while the linear species were always absent. This result finds explanation only in the absence of positively charged groups on the porphyrin skeleton. Indeed, although in the case of catalyst **Mn-5** the steric hindrance of the long side chain can be considered as further limitation of the activity, it is not the case of the other three catalysts. In particular, porphyrins with electron withdrawing chlorine atoms and lipophilic sulphonamido side chains proved to be active in alkene epoxidations.[43] This means that catalysts **Mn-(5–8)** fulfil the structural requirements necessary to give the high valent metallo-oxo species, the 'true' oxidant, hence the lack of activity can be only imputed to the absence of any interaction between DNA and catalyst.

This statement was confirmed by investigating the demolition of plasmid Bluescript promoted by visible light in the presence of both Zn- and Mn-complexes of porphyrins **H_2-9** and **H_2-21** [**Zn-(1,8)** and **Mn-(1,8)** respectively] which, in this case, act as photosensitizers; under photoactivation conditions, singlet oxygen becomes the oxidising species, generated from molecular oxygen by energy transfer from the macroring in its excited triplet state. Hence, irradiating a 10^{-7} M Plasmid solution (3×10^{-4} M in base pair) containing **Zn-1 (Mn-1)** or **Zn-8 (Mn-8)** catalysts (3×10^{-6} M) for three h with a slide projectors (placed at 45 cm from the wells, fitted with a 150 W lamp and an infrared glass filter), the partial demolition of Bluescript was observed. As it was found in the presence of oxygen donors, the activity of the 'charged' catalyst was, by far, higher than that of the lipophilic one, being the Zn-complexes more active than the Mn derivative; this last observation is in agreement with the knowledge that paramagnetic metal complexes are not suitable for PDT. While both metal complexes **Zn-8** and **Mn-8** were found totally inert, catalyst **Zn-1** gave a complete demolition of the plasmid with the formation of 40 and 60% of nicked and linear forms, respectively. Although these results confirm the reactivity observed in the presence of oxygen donors, that is charged catalysts are active while uncharged are not, the absence of activity of catalyst **Zn-8** was quite surprising in view of the structural affinity of catalyst **Zn-8** with the tetra(3-hydroxyphenyl)chlorin (trade name Foscan), which is a promising molecule for the photodynamic therapy of tumour (PDT),[44] that has recently received an EDR approved pharmaceutical.

This intriguing result induced us to carry out an ancillary experiment on the activity of **Zn-1** and **Zn-8** on tumour cells. Aqueous solution of **Zn-1** and **Zn-8** (in the range 1.0–0.01 μM), were added to a culture of HTC116 cells in D-MEM and incubated for 24 h. After this period the medium was changed with PBS and sample irradiated for 3 h with a slide projector; the original medium was then replaced and cell activity assessed with MTT assay after 24 h. In this experiment, the activity/structure relationship of the catalysts was the opposite to what found on Bluescript plasmid, indeed **Zn-1**, featuring ionic substituents, was found completely inactive while the lipophilic catalyst (**Zn-8**) produced an IC_{50} at 5×10^{-2} μM concentrations. At this stage of the research, we cannot recognize if necrosis or apoptosis are involved in cells death.

The results of this work confirm that metallo-tertaarylporphyrins can be used as catalysts for oxidative demolition of both supercoiled DNA and whole cells, either in the presence of an artificial oxygen donor or molecular oxygen/hν as primary oxidant; however, the structural characteristic of the catalysts are crucial to achieve reactivity on poly-nucleotides or on cells. The presence of positively charged groups on the porphyrin skeleton seems fundamental in Mn-porphyrins DNA catalysed demolition probably via electrostatic interactions; in particular, the presence of 4-methylpyridinium rings on the *meso* porphyrin positions confers the highest activity among all the aryl substituents considered in this work. An explanation could be envisaged on the accessibility of the positive charge on the methyl pyridinium group in comparisons with that on the sterically demanding trimethylammonium group.

On the other hand, the presence of four ionic groups on the periphery of the porphyrin skeleton has the opposite effect, inhibiting the interaction with cells. In order to achieve a remarkable activity on cells, lipophilic substituents are necessary to obtain a good cell penetration; however extremely lipophilic photosensitizers are hardly soluble in physiological aqueous phase thus their administration become troublesome. A compromise between hydrophilic and lipophilic character of the photosensitizers has to be reached for an in vivo application, indeed amphiphilicity is commonly recognized as the fundamental property of photosensitizers since both biodistribution and selectivity for tumour over healthy tissues were attributed to such characteristic of the molecules.[16,45] On the base of these observations, the design of newly structured photosensitizers, featuring a tetrapyrrole skeleton, is currently under way.

Experimental

General

UV–visible absorption spectra were measured on a Perkin-Elmer Lambda 10 instrument. ^{1}H NMR spectra

were recorded on a Bruker 400 MHz spectrometer in $CDCl_3$ or [d_6] DMSO; chemical shifts are expressed in ppm relative to chloroform (7.26). Mass spectrometric measurements were performed on a Finnigam LQC-MS with electronspray ionization (ESI) or atmospheric pressure chemical ionization (APCI) ion sources, fitted with an ion trap mass analyzer.

MALDI-TOF-MS experiments: each sample was dissolved in methanol to a final concentration of about 100 mM and mixed 1:1 with a saturated solution of the matrix (α-ciano-4-hydroxycinnamic acid or 2,5-dihydroxybenzoic acid in 50% acetonitrile). 0.5 mL was spotted directly on a MALDI target plate. MALDI mass spectra were acquired in the positive reflectron ion mode with delayed extraction on a Reflex III time of flight instrument (Bruker Daltonics, Bremen, Germany) equipped with a 337 nm nitrogen laser. Ion acceleration voltage was set to 25.00 kV, the reflector voltage was set to 28.70 kV. Mass spectra were obtained by averaging 200 laser shot.

HPLC analyses were conducted with a Thermo Separation Products (TSP) instrument coupled with a Finnigan LCQ-MS with electron spray (ESI) or atmospheric pressure chemical ionization (APCI) ion source. The instrument was fitted with a 0.21×15 cm column (Supelco, Discovery) packed with C-18 reversed-phase particles (5 μm) and operated with an isocratic elution with A/B 30:70 ratio (A = H_2O, 0.1% CH_3COOH; B = CH_3CN/CH_3OH 80:20) at 0.2 mL/min.

Analytical thin-layer chromatography (TLC) was performed using Merck 60 F254 silica gel (precoated sheets, 0.2 mm thick) or on Macherey-Nagel F254 silica gel C_{18}-100 (precoated sheets, 0.25 mm thick). Silica gel 60 (70–230 mesh, Merck) was used for column chromatography. Buffer solutions were prepared as follows.

pH 10.4: 1 L of buffer was obtained diluting 11.2 mL of a 1.0 M solution of $NaHCO_3$ and 29.5 mL of a 1.0 M solution of Na_2CO_3; *pH 9.2*: 1 L of buffer was obtained diluting 76.8 mL of a 1.0 M solution of $NaHCO_3$ and 7.74 mL of a 1.0 M solution of and Na_2CO_3; *pH 5.7*: 0.1 L of buffer was obtained diluting 50 mL of a 0.1 M solution of potassium hydrogenphthalate and 25.5 mL of a 0.1 M solution of NaOH.

5,10,15,20-Tetra(4-hydroxyphenyl)-21H,23H-porphyrin [H_2-T(4-OHP)P, **H_2-21**], *5,10,15,20*-Tetra[4-(trimethylammonio)phenyl]-21H,23H-porphyrin tetra-*p*-tosylate salt [H_2-T(4-$Me_3N^+Ts^-$P)P, **H_2-10**] and *5,10,15,20*-Tetra(1-methyl-4-pyridyl)-21H,23H-pophyrin tetra-*p*-tosylate salt [H_2-T(4-$MePy^+Ts^-$)P, **H_2-9**] were commercial products.

Synthesis of free-base tetraarylporphyrins

5,10,15,20-Tetraphenyl-21H23H-porphyrin [H_2-TPP, **H_2-18**], *5,10,15,20*-Tetra(2,6-dichlorophenyl)-21H,23H-porphyrin [H_2-T(2,6-Cl_2P)P, **H_2-14**] and *5,10,15,20*-Tetra(2,6-dichloro-3-nitrophenyl)-21H,23H-porphyrin [H_2-T(2,6-Cl_2-3-NO_2P)P, **H_2-11**][36] were synthesized via condensation of the corresponding aromatic aldehydes and pyrrole, according to the procedure describe by Lindsey.[46] The chlorosulphonyl derivative of H_2-TPP (**H_2-19**) and of H_2-T(2,6-Cl_2P)P (**H_2-15**) were obtained by the reaction of free-base porphyrins with chlorosulphonic acid following the method described by Rocha Gonsalves.[42] Catalyst **Mn-5** was synthesized as reported in our previous work.[43]

***5,10,15,20*-Tetra (2,6-dichloro-3-aminophenyl)-21H,23H-porphyrin (H_2-12).** This compound was synthesized following a modified procedure as to the previously reported.[35] To a solution of 200 mg (1.9×10^{-1} mmol) of *meso*-tetra(2,6-dichloro-3-nitrophenyl)-21H,23H-porphyrin in 50 mL of AcOEt were added 422 mg of $SnCl_2\times2H_2O$ (1.9 mmol) and the mixture was kept at 70 °C for 18 h. After cooling, 20 mL of aqueous Na_2CO_3 saturated solution were added, the phases were separated and the organic layer was thoroughly washed with water and dried (Na_2SO_4). The solvent was evaporated and the row material purified by column chromatography (SiO_2, CH_2Cl_2/CH_3OH 98/2) affording 167 mg (1.8×10^{-1} mmol; 93% yield) of the desired aminoporphyrin ($C_{44}H_{26}N_8Cl_8$, M_r = 950.4). TLC (SiO_2; CH_2Cl_2/CH_3OH: 98/2) R_f = 0.70; UV–vis$_{(dichloromethane)}$: λ_{max} 418 nm (ε = 212,000 mol^{-1} L cm^{-1}). ^1H NMR (DMSO-d_6) δ = −2.75 (s, 2H), 5.78–5.82 (br s, 8H), 7.35 (d, 4H), 7.66 (d, 4H), 8.70–8.80 (br s, 8H). MS (MALDI-TOF): m/z 950.7 [$(M+H)^{]+}$]; 916.7 [$(M-Cl+H)^{]+}$].

***5,10,15,20*-Tetra(2,6-dichloro-3-potassiumsulphonatophenyl)-21H,23H-porphyrin (H_2-16).** The *5,10,15,20*-Tetra(2,6-dichloro-3-chlorosulphonylphenyl)-21H,23H-porphyrin (**H_2-15**) ($C_{44}H_{18}N_4Cl_{12}O_8S_4$, M_r = 1284.3) was synthesised stirring for 2 h a solution of *5,10,15,20*-tetra(2,6-dichlorophenyl)-21H,23H-porphyrin in chlorosulfonic acid at 100 °C, following the procedure described by Rocha Gonsalves,[42] and recovered in 60% yield, after crystallization from $CHCl_3$/*n*-hexane. Afterwards, 43 mg (3.4×10^{-2} mmol) of the chlorosulphonyl derivative was hydrolyzed to the corresponding tetrapotassium sulphonate with 18.5 mg (1.3×10^{-1} mmol) of K_2CO_3 in wet acetone, to give 45 mg (78%) of the title compound. ($C_{44}H_{18}Cl_8N_4O_{12}S_4K_4$, M_r = 1362.8). TLC (RP-C18, CH_3CN/H_2O 9/1) R_f = 0.8; UV–vis$_{(water)}$: λ_{max} 414 nm (ε = 221,000 mol^{-1} L cm^{-1}). ^1H NMR (D_2O) δ = 7.95 (m, 4H), 8.45 (d, 4H), 8.94 (br s, 8H). MS (MALDI-TOF): m/z 1210.4 [$(M-4K+4H)^{]+}$] (the acid form of the compound was generated by the MS matrix; a low intensity signal corresponding to mono-potassium salt was also present).

***5,10,15,20*-Tetra(2,6-dichloro-3-N-ethoxyethanolsulphonamidophenyl)-21H,23H-porphyrin (H_2-17).** The **H_2-15** porphyrin was synthesized as reported above and immediately made to react with an excess of 2-(2-aminoethoxy)ethanol (AEE); thus, to a CH_2Cl_2 solution (7 mL) of 58 mg (4.5×10^{-2} mmol) of **H_2-15** was added 0.1 mL (9.8×10^{-1} mmol) of AEE and 0.1 mL of Et_3N; the mixture was kept at 40 °C for 2 h, then at RT for 15 h. The organic phase was washed with portion of slightly acidic water, to eliminate the ammonium salts and the

excess of AEE, dried over Na_2SO_4 and evaporated to dryness. The residue was purified through column chromatography (SiO_2, CH_2Cl_2/MeOH 9/1) to give 30 mg (42% yield) of the title compound. ($C_{60}H_{58}Cl_8N_8O_{16}S_4$, $M_r = 1557.3$). TLC (SiO_2; CH_2Cl_2/CH_3OH 7/3) $R_f = 0.85$; UV–vis$_{(methanol)}$: λ_{max} 414 nm ($\varepsilon = 223{,}000\ mol^{-1}\ L\ cm^{-1}$). ^1H NMR ($CDCl_3$ + DMSO-d_6) $\delta = -2.62$ (s, 2H), 3.30 (m, 8H), 3.40 (m, 20H), 3.55 (m, 8H), 4.72 (br s, 4H), 7.94 (m, 4H), 8.51(m, 4H), 8.58 (br s, 8H). MS (MALDI-TOF): m/z 1558.4 [$(M+H)^{]+}$].

***5,10,15,20*-Tetra(4-*N*-ethoxyethanolsulphonamidophenyl)-21H,23H-porphyrin (H_2-20).** The *meso*-Tetra(4-chlorosulphonylphenyl)-21H,23H-porphyrin was synthesized following a slightly modified method as described by Rocha Gonsalves,[42] indeed at room temperature only a partial sulphonation of the tetraphenyl-porphyrin was observed, hence the reaction with chlorosulphonic acid was carried out at 60 °C for 1 h. The chlorosulphonyl intermediate (**H_2-19**) was then immediately made to react with an excess of 2-(2-aminoethoxy)ethanol (AEE); thus, to a CH_2Cl_2 solution (25 mL) of 169 mg (1.6×10^{-1} mmol) of **H_2-19** were added 1 mL (9.8 mmol) of AEE and 0.1 mL of Et_3N; the mixture was kept at 40 °C for 2 h. The desired product precipitated on cooling and recovered by filtration (141 mg). The row material was crystallized from a mixture of CH_2Cl_2/hexane to give 120 mg of the pure product (60% yield; $C_{60}H_{66}N_8O_{16}S_4$, $M_r = 1283.5$). TLC (SiO_2; CH_2Cl_2/CH_3OH 9/1) $R_f = 0.35$; UV–vis$_{(methanol)}$: λ_{max} 414 nm ($\varepsilon = 248{,}000\ mol^{-1}.L.cm^{-1}$); ^1H NMR (DMSO-d_6) $\delta = -2.94$ (s, 2H), 3.26 (t, 8H), 3.49 (m, 8H), 3.55 (m, 20H), 4.66 (br s, 4H), 8.25 (d, 8H), 8.251(d, 8H), 8.45 (s, 8H). MS (MALDI-TOF): m/z 1282.9 [$(M)^{]+}$].

General procedure for the preparation of tetraarylporphyrin metallo-complexes

The metallo-porphyrin derivatives were obtained, following a general method described by Adler,[47] refluxing dimethylformamide (DMF) or water solutions, for neutral H_2-porphyrins or ionic H_2-porphyrins respectively, in the presence of an excess of the desired salt. The reaction progress was determined monitoring the visible spectra changes of the porphyrin Soret band from the 418–422 nm of the free-base macrocycle to the 460–478 nm (the wavelength depends on the solvent used) of the Mn-complexes. The formation of the Zn-derivatives was determined at 438 nm.

***5,10,15,20*-Tetra(1-methyl-4-pyridyl)porphyrin tetrachloride salt Mn(III) chloride complex. [Mn-1].** A water solution (10 mL) of 91 mg of **H_2-9** porphyrin ($M_r = 1363.6$; 6.7×10^{-2} mmol) was treated with 245 mg (1.0 mmol) of manganese acetate tetrahydrate under reflux for 6 h. The mixture was cooled and the Mn–porphyrin precipitated by the addition of a solution of $NaClO_4$ (3 g in 3.5 mL of H_2O); the solid residue was recovered by filtration, dissolved in methanol and filtered a second time to eliminate insoluble material. The anion (ClO_4^-) was then exchanged with Cl^- treating the solution with amberlit IRA-400 chloride form; the **Mn-1**, 55 mg (90% yield), was recovered as a dark green solid by addition of diethylether to the filtered methanol solution. ($C_{44}H_{36}Cl_5N_8Mn$, $M_r = 909.0$), UV–vis$_{(water)}$: λ_{max} 462 nm ($\varepsilon = 97{,}800\ mol^{-1}\ L\ cm^{-1}$). TLC (any condition $R_f = 0$). HPLC: Retention time = 2.01 min. MS (APCI$^+$): m/z 663.3 [(M + H)-5Cl-Mn-$CH_3^{]+}$].

***5,10,15,20*-Tetra(1-methyl-4-pyridinio)porphyrin tetrachloride Zn(II) complex. [Zn-1].** This catalyst was obtained in quantitative yield from the commercial *5,10,15,20*-tetra(1-methyl-4-pyridyl)-21H,23H-porphyrin tetra-*p*-tosylate salt (**H_2-9**), refluxing a porphyrin water solution for 3 h in the presence of an excess of $ZnCl_2$. The reaction mixture was then worked up as described above for the Mn-complex. **Zn-1**: $C_{44}H_{36}Cl_4N_8Zn$, $M_r = 883.6$; UV–vis$_{(water)}$: λ_{max} 438 nm ($\varepsilon = 163{,}200\ mol^{-1}\ L\ cm^{-1}$). ^1H NMR (DMSO-d_6) $\delta = 4.72$ (br s, 12H), 8.84 (d, 8H), 9.01(s, 8H), 9.48 (d, 8H). MS (APCI$^+$): m/z 849.4 [$(M-Cl+H)^{]+}$].

***5,10,15,20*-Tetra[4-(trimethylammonio)phenyl]porphyrin tetrachloride Mn(III) chloride complex (Mn-2).** A solution of 39 mg (2.5×10^{-2} mmol) of **H_2-10** in 15 mL of H_2O was refluxed for 5 h in the presence of 61 mg (2.5×10^{-1} mmol) of the manganese salt. The manganese complex was precipitated as penta-perclorate salt by the addition of 3.0 g of $NaClO_4$ (3.5 mL aqueous solution) and recovered after filtration, yielding 30 mg (2.1×10^{-2} mmol, 86%) of porphyrin ($C_{56}H_{60}Cl_5N_8O_{20}Mn$ $M_r = 1397.3$), UV–vis$_{(water)}$: λ_{max} 464 nm ($\varepsilon = 63{,}000\ mol^{-1}\ L\ cm^{-1}$). The counter anions were exchanged stirring a porphyrin methanol solution in the presence of amberlit IRA-400 chloride form to give the desired compound in quantitative yield. **Mn-2**: $C_{56}H_{60}Cl_5N_8Mn$, $M_r = 1077.3$. UV–vis$_{(water)}$: λ_{max} 462 ($\varepsilon = 71{,}000\ mol^{-1}\ L\ cm^{-1}$). HPLC: retention time = 1.71 min. MS (APCI$^+$): m/z 840.5 [$(M-5Cl-4CH_3)^{]+}$].

***5,10,15,20*-Tetra(2,6-dichloro-3-aminophenyl)porphyrin manganese(III) chloride complex (Mn-13).** A DMF solution of **H_2-12** 152 mg (8.8×10^{-1} mmol) was refluxed in the presence of 492 mg (2.0 mmol) of Mn(OAc)$_2$·4H$_2$O for 6 h; the porphyrin **Mn-13** (33 mg, 3.2×10^{-1} mmol, 36% yield) was recovered after column chromatography (SiO_2, CH_2Cl_2/CH_3OH 9/1) and crystallization from a mixture of dichloromethane/hexane. ($C_{44}H_{24}Cl_9N_8Mn$, $M_r = 1038.7$), UV–vis$_{(methanol)}$: λ_{max} 466 nm ($\varepsilon = 95{,}000\ mol^{-1}\ L\ cm^{-1}$). TLC ($SiO_2$, $CHCl_3$/*n*-hexane: 8/2) $R_f = 0.35$. HPLC: retention time = 2.63 min. MS (ESI$^+$): m/z 1003.4 [$(M-Cl)^{]+}$]

***5,10,15,20*-Tetra(2,6-dichloro-3-potassiumsulphonatophenyl)porphyrin-Mn(III) chloride complex (Mn-4).** The manganese derivative was synthesized refluxing an aqueous solution of 45 mg (2.6×10^{-1} mmol) of porphyrin **H_2-16** for 3 h in the presence of Mn-acetate. Compound **Mn-4** was recovered in 74% yield (28 mg, 1.9×10^{-1} mmol) after filtration on reversed-phase silica (RP-C_{18}) and subsequent column chromatography (SiO_2, CH_3CN/2-propanol 1/1). $C_{44}H_{16}N_4Cl_9O_{12}S_4K_4Mn$, $M_r = 1451.3$; UV–vis$_{(water)}$: λ_{max} 464 nm ($\varepsilon = 99{,}000\ mol^{-1}\ L\ cm^{-1}$). TLC (RP-$C_{18}$, CH_3CN) $R_f = 0.64$. HPLC: retention time = 1.51 min. MS (ESI$^-$): m/z 420.1 [$(M-4K-Cl)/3^{]-}$].

***5,10,15,20*-Tetra(2,6-dichloro-3-*N*-ethoxyethanolsulphonamidophenyl) - porphyrin - Mn(III) chloride complex (Mn-6).** From 26 mg of a DMF solution (15 mL) of porphyrin **H_2-17**, the manganese complex (**Mn-6**) was obtained in 71% yield (19 mg) after column chromatography (SiO_2, AcOEt/EtOH 9/1–8/2). **Mn-6**: $C_{60}H_{56}Cl_9N_8O_{16}S_4Mn$, $M_r = 1647.4$; UV–vis$_{(methanol)}$: λ_{max} 464 nm ($\varepsilon = 86{,}000$ mol^{-1} L.cm^{-1}) TLC (SiO_2, $CHCl_3/CH_3OH$ 7/3) $R_f = 0.75$. HPLC: retention time = 1.61 min. MS (ESI$^+$): m/z 1611.8 [(M−Cl)$^{]+}$].

***5,10,15,20*-Tetra(4-N-ethoxyethanolsulphonamidophenyl)-porphyrin-Mn(III) chloride complex (Mn-7).** Metallation of porphyrin **H_2-20** with manganese was achieved in DMF at 140 °C for 6 h; compound **Mn-7** was obtained in about 90% yield after column chromatography (SiO_2, AcOEt/EtOH 8/2–6/4) and AcO^- counter anion exchange with Cl^-. $C_{60}H_{64}ClN_8O_{16}S_4Mn$, $M_r = 1371.8$; UV–vis$_{(methanol)}$: λ_{max} 468 nm ($\varepsilon = 68{,}000$ mol^{-1} L cm^{-1}). TLC (SiO_2, $CHCl_3/CH_3OH$ 7/3) $R_f = 0.80$. HPLC: retention time = 1.45 min. MS (ESI$^+$): m/z 1335.8 [(M−Cl)$^{]+}$].

***5,10,15,20*-Tetra(2,6-dichloro-3-trimethylammoniophenyl)-porphyrin tetra-chloride salt Mn(III) chloride complex (Mn-3).** Porphyrin **Mn-13** (33 mg, 3.2×10^{-2} mmol) was treated with 3 mL (31.6 mmol) of dimethylsulphate (M_r 126, $d = 1.33$ g/mL) and 0.1 g of solid K_2CO_3 at 150 °C for 6 h. After this period, 10 mL of water were added and the mixture washed 5 times with 10 mL of CH_2Cl_2 in order to eliminate the excess of dimethylsulphate. The aqueous phase was then evaporated under vacuum. The counter anions were exchanged stirring a porphyrin methanol solution in the presence of amberlit IRA-400 chloride form to give the desired compound that it was dissolved in ethanol and precipitated with hexane to give 19 mg of **Mn-3** (1.4×10^{-2} mmol, 44.2% yield). ($C_{56}H_{52}Cl_{13}N_8Mn$, $M_r = 1352.9$), UV–vis$_{(water)}$: λ_{max} 464 nm ($\varepsilon = 75{,}000$ mol^{-1} L cm^{-1}).TLC (any condition $R_f = 0$). HPLC: retention time = 2.17 min. MS (ESI$^+$): m/z 1115.1 [(M−5Cl−4CH$_3$)$^{]+}$].

It has to be noted that a partial porphyrin demetallation was observed in this reaction thus, in some cases, the treatment with manganese salts had to be repeated after methylation.

Metal complexes of *5,10,15,20*-Tetra(4-hydroxyphenyl)-21H,23H-porphyrin: Mn-8 and Zn-8. The title compounds were synthesized under standard conditions from the commercial porphyrin (**H_2-21**) and $Mn(AcO)_2$ or $Zn(AcO)_2$ salts. **Zn-8** was obtained in 92% yield after column chromatography (SiO_2, AcOEt/CH_2Cl_2/MeOH 4/5.8/0.2); $C_{44}H_{28}N_4O_4Zn$, $M_r = 742.1$; UV–vis$_{(DMSO)}$: λ_{max} 430 nm ($\varepsilon = 138{,}000$ mol^{-1} L cm^{-1}). TLC (SiO_2, CH_2Cl_2/CH_3OH 9/1) $R_f = 0.58$. ^{1}H NMR ($CDCl_3$ + DMSO-d_6) $\delta = 7.13$ (d, 8H), 7.91 (d, 8H), 8.82 (s, 8H), 9.19 (s, 4H). MS (ESI$^+$): m/z 741.4 [(M)$^{]+}$]. **Mn-8** was obtained in 73% yield after column chromatography (SiO_2, AcOEt/CH_2Cl_2/MeOH 3/3/4); $C_{44}H_{28}ClN_4O_4Mn$, $M_r = 767.1$; UV–vis$_{(DMSO)}$: λ_{max} 470 nm, ($\varepsilon = 68{,}000$ mol^{-1} L cm^{-1}). TLC (SiO_2, CH_2Cl_2/CH_3OH 9/1) $R_f = 0.38$. HPLC: retention time = 1.78 min. MS (ESI$^+$): m/z 731.6 [(M−Cl)$^{]+}$]

Oxidative cleavage of Plasmid Bluescript

The plasmid was isolated from a culture of *Escherichia coli* with the aid of Genomed extraction kit. After each extraction, 300 μL of water solution were obtained, with a concentration of about 200 ng/μL [3×10^{-4} M (bp)] of plasmid, as spectroscopically assessed by measuring the absorbance of DNA solution at 260 nm.

Effect of the concentration of the oxygen donor and pH. In order to ensure at least three 10-μL samples withdrawing, the reaction solutions were prepared as follows: to 21 μL of the desired buffer (Table 1) were added 7 μL of Bluescript solution (3×10^{-4} M) and 7 μL of oxygen donor ([OD] = 3×10^{-4} to 3×10^{-1} M) in order to achieve the desired ratio with respect to the DNA. The final concentration of plasmid is [DNA] = 40 ng/μL; 6.06×10^{-5} M. Samples of 10 μL of this solution were withdrew at different times, then 3 μL of sodium tiosulfate (0.103 N) were added to each sample, as quenching agent for the excess of oxidant, followed by 2 μL of bromophenol blue. The mixture was then loaded into the wells of a 1.0% horizontal agarose gel (incubated with ethidium bromide) and analyzed at 110 mV. A plasmid buffer solution was used as reference. The DNA bands were detected by fluorescence under UV lamp; a correction factor of 1.5 was used for the intensity of the nicked and linear Plasmid with respect to the one of the supercoiled form.[35] The results are reported in Table 1.

Effect of the concentration of Mn-1 porphyrin. The reaction solution was prepared as described above, while replacing the OD solution with that one of catalyst **Mn-1**. Samples of 10 μL were collected at different times and treated with a reducing agent and with bromophenol blue then charged on agarose gel. The results of the electrophoresis are reported in Table 2.

Catalytic effect of the system Mn-porphyrin/OD. For these experiments, the reaction solutions were prepared as follows: 7 μL of a 3×10^{-4} M plasmid solution, 7 μL of Mn-porphyrin, 7 μL of OD solution (these last were used at such concentration to get the desired ratio with respect to the DNA) and 21 μL of buffer. The samples of 10 μL were processed as described above and the results are reported in Tables 3–5.

Cell cultures

Human colon adenocarcinoma cells (HCT116) were used. HCT116 were grown in 25 cm^2 flasks and mantained in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum (FCS), 2% L-glutamine and 1% penicillin–streptomycin. HCT116 were cultivated in a humidified atmosphere af 5% CO_2 and 95% air. Cells usually were reseeded into fresh medium every 2 days.

Phototoxicity

About 5×10^3 HCT116 cells per well in 100 μL DMEM 10% FCS, were left 2 days (48 h, 37 °C, 5% CO_2) in a

96-well plate. The FCS solution was supplemented with 1% L-glutamine and 1% penicillin–streptomicyn. The first column (eight wells) served as a blank without cells. The second served as control, without dyes (100% cell survival). In another column, HCT116 were incubated with 100 μL of the different porphyrins preparations (1 mg/mL in DMSO), diluted in DMEM, at 37 °C, in a dark, humid atmosphere containing 5% CO_2. After 24 h, DMEM were substituted with PBS and exposed at room temperature to 500-W halogen lamp fitted with aqueous filter at graded fluences of 0–860 mJ cm^{-2} s^{-1} for 2 h. After the phototreatment, the cells were incubated overnight with 200 μL DMEM as described above.

Cell survival was then assessed by means of the colorimetric MTT assay;[48–50] 50 μL of the MTT solution at 2% in PBS were added to all wells followed by 3 h incubation at 37 °C. After, the 96-well plates were centrifugated for 10 min at 2000 rpm, Formazan crystals were solubilized with 120 μL of DMSO and the assorbance at 570 nm was read on a Universal Microplate Reader (Bio-Teck Instruments).

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