Bioorganic & Medicinal Chemistry 17 (2009) 767–776

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

DNA photocleavage by porphyrin–polyamine conjugates

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ARTICLE INFO

Article history:
Received 28 August 2008
Revised 13 November 2008
Accepted 15 November 2008
Available online 25 December 2008

Keywords:
Porphyrin
Polyamines
DNA photocleavage
Singlet oxygen

ABSTRACT

A series of polyamine–porphyrin conjugates bearing two (*cis* or *trans* position) or four units of spermidine or spermine was synthesized. We studied the binding of these cationic porphyrins to calf thymus DNA by the means of UV–vis spectroscopy and we investigated their ability to cleave plasmid DNA in the presence of light. DNA binding and DNA photocleavage abilities were found to depend on structural characteristics as (a) the relative positions of the side chains on the porphyrin ring and (b) the nature of the attached side chains (spermidine or spermine). DNA cleavage was also studied in the presence of a singlet oxygen quencher (NaN_3) and in the presence of a hydroxyl radical scavenger (mannitol). Singlet oxygen was the major species responsible for the cleavage of DNA previously observed. Collectively, these data show that polyamine–porphyrin conjugates could be promising phototherapeutic agents.

1. Introduction

Photodynamic therapy (PDT) is a type of cancer therapy based on the selective retention of photosensitizers such as porphyrins in tumours.[1] Upon irradiation, these photosensitizers cause irreversible photodamage to malignant cells.[2] However the poor selectivity of photosensitizing drugs frequently leads to the necrosis of surrounding healthy tissues together with a skin photosensivity that may last several weeks after treatment. Selective targeting of cancer cells thus appears essential to circumvent these problems and to improve PDT efficiency.[3] Porphyrin polyamine conjugates could present one of the ways to meet these goals.

Polyamines such as spermine, spermidine and putrescine, are required for cellular growth[4] and their concentrations are especially elevated in rapidly proliferating cells. Cancer cells, whose polyamine requirements exceed their biosynthetic capabilities, use a polyamine transport system (PAT) to fulfil their needs. This system displays at the same time a strong affinity and a loose specificity. Therefore, the polyamine transport system affords a selective accumulation of polyamine analogues in neoplastic tissues and consequently plays a very attractive role in several anticancer chemotherapeutic strategies.[5] In connection with our research program on PDT and targeted porphyrin derivatives,[6] we presented in a previous article the synthesis of a protoporphyrin polyamine conjugate which proved highly cytotoxic at the sub-micromolar level.[7] The understanding of porphyrin polyamine toxicity implies the knowledge of their intracellular targets. Nucleic acids are first rank targets of cationic porphyrins, since the latter can intercalate into DNA and contract additional electrostatic bonding with the negatively charged phosphodiester backbone.[8] The formation of these complexes greatly helps PDT-induced damage to DNA.

This paper described the design and the synthesis of a new series of four porphyrins bearing two or four polyamine molecules (spermine or spermidine) attached by the means of a flexible arm. We studied the interaction of these molecules with calf thymus DNA along with the photocleavage of plasmid pBR322.

2. Chemistry

2.1. Synthesis

N-Boc-Spermine and *N*-Boc-spermidine derivatives **1** and **2** (Scheme 2) were synthesised from spermine and spermidine, respectively, as described in a previous paper.[9] *meso*-Tetrakis(4-carboxyphenyl)porphyrin **3** (TCPP, Scheme 2) was synthesized by the Little method; condensation of pyrrole with *para*-carboxybenzaldehyde in stoichiometric amounts in propionic acid gave the expected porphyrin in 16% yield.[10] 5, 15-Bis(4-carboxyphenyl)10,20-bis(mesityl)porphyrin **6** was synthesized according to Scheme 2; condensation of *meso*-(mesityl)dipyrromethane **4** (1 equiv) with 4-formylmethylbenzoate (1 equiv) in TFA gave, after oxidation by *p*-chloranil and column chromatography, porphyrin derivative **5** in 30% yield. This compound was converted into derivative **6** in excellent yield (94%) by saponification with KOH/EtOH (1 M in DMF) at reflux for 45 min. Condensation of TCPP **3** with polyamine

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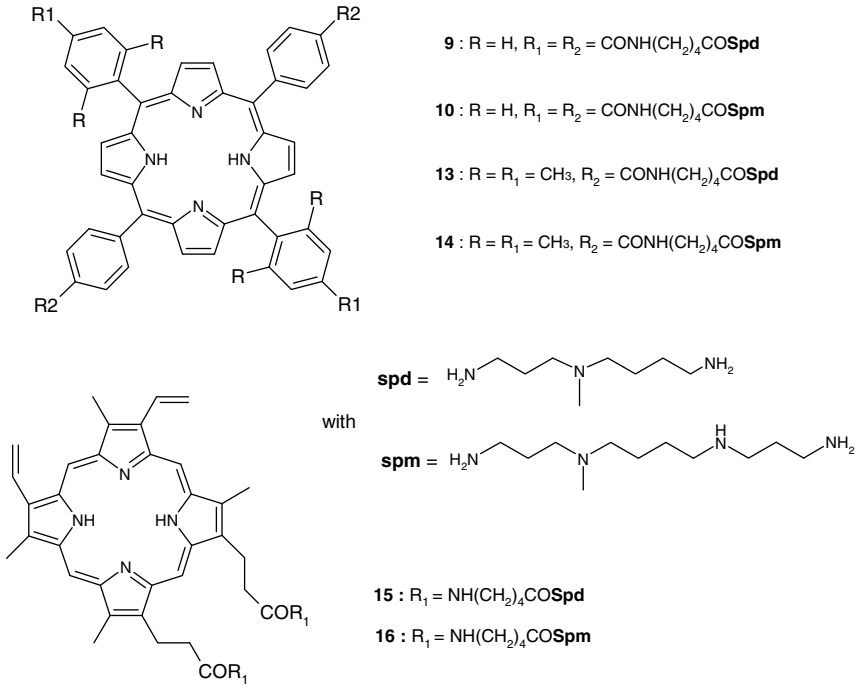
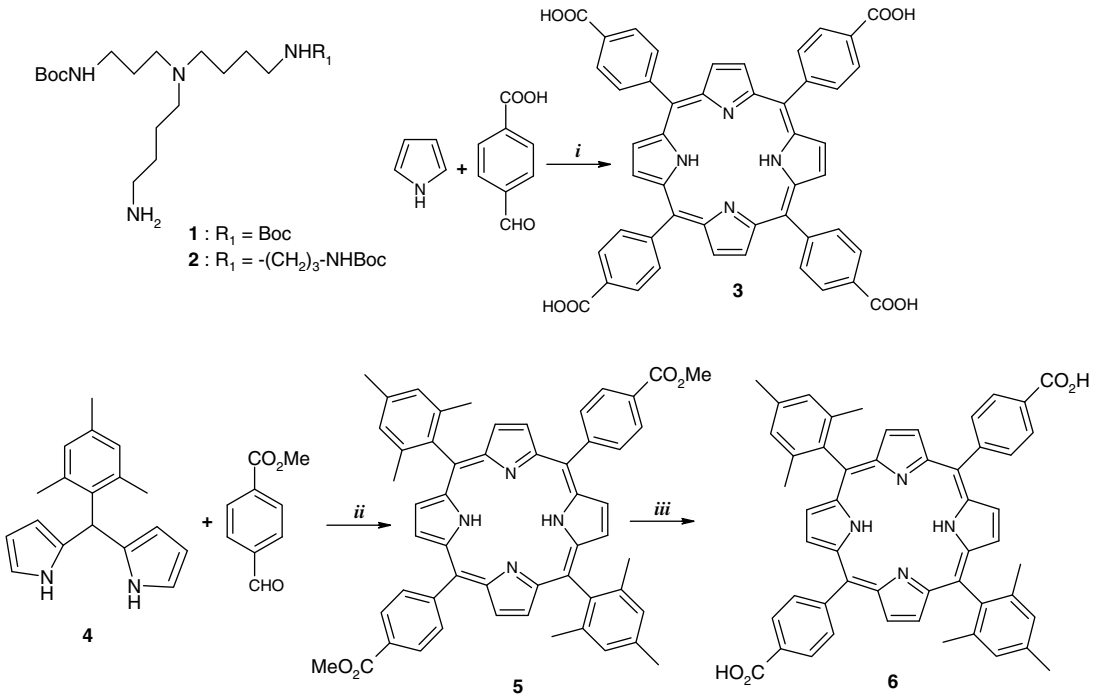
0968-0896/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmc.2008.11.047



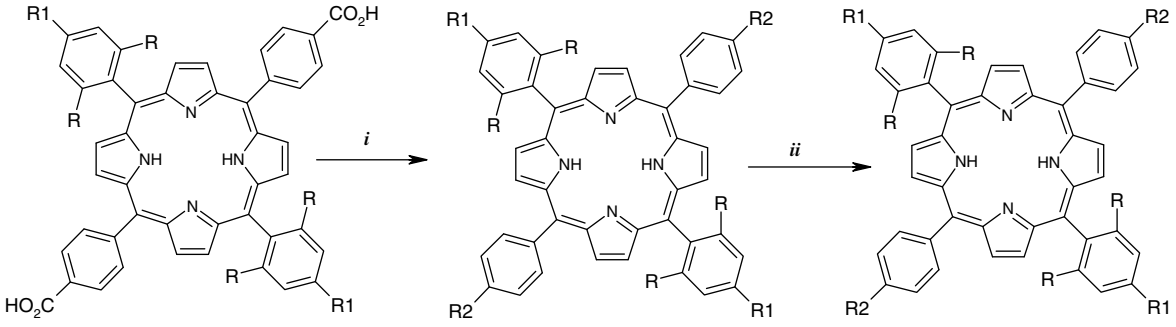
Scheme 1. Structures of six porphyrin polyamine derivatives.



Scheme 2. Structure of synthons for synthesis of porphyrins polyamines derivatives (i). Propionic acid, reflux, 90 min, 16%, (ii) (a) TFA (1 equiv), CH_2Cl_2, 1 h 30, rt, (b) *p*-chloranil, reflux, 1 h, 30% (iii) KOH/EtOH, 1 M, 45 min, reflux, 94%.

derivative, either **1** or **2**, was realized in presence of *N*,*N*′-dicyclocarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in dry DMF, at room temperature for 18 h. After purification by TLC ($CHCl_3$/EtOH: 7/3 + 2% Et_3N), protected polyamine porphyrin conjugates **7** and **8** were obtained in 42% and 51% yields, respectively. Finally porphyrin derivatives **9** and **10** were obtained in quantitative yields after deprotection by treatment with TFA/CH_2Cl_2 (1/1) at room temperature for 2 h.[11]

In the same conditions, 5,15 dimesityl 10,20-(4-carboxyphenyl)porphyrin **6** (Scheme 3) reacted with N^4-(4-aminobutyl)-N^1,N^8-bis-*tert*-butoxycarbonylspermidine or N^4-(4-aminobutyl)-N^1,N^8,N^{12}-tris-*tert*-butoxycarbonylspermine in presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in DMF. After purification by TLC (eluent: $CHCl_3$/EtOH 70:30 + 1.5% Et_3N), protected polyamine porphyrin conjugates **11** and **12** were obtained in 73% and 68% yields, respectively. After cleavage of



3 : R = H, R_1 = COOH

6 : R = R_1= CH_3

7 : R = H and R_1 = R_2 = $CONH(CH_2)_4CO$**Spd**$(Boc)_2$

8 : R = H and R_1 = R_2 = $CONH(CH_2)_4CO$**Spm**$(Boc)_3$

9 : R = H and R_1 = R_2 = $CONH(CH_2)_4CO$**Spd**

10 : R = H and R_1 = R_2 = $CONH(CH_2)_4CO$**Spm**

11: R = R_1 = CH_3 and R_2 = $CONH(CH_2)_4CO$**Spd**$(Boc)_2$

12 : R = R_1 = CH_3 and R_2 = $CONH(CH_2)_4CO$**Spm**$(Boc)_3$

13 : R = R_1 = CH_3 and R_2 = $CONH(CH_2)_4CO$**Spd**

14 : R = R_1 = CH_3 and R_2 = $CONH(CH_2)_4CO$**Spm**

Scheme 3. Reagents and conditions: (i) DCC (10 equiv), HOBt (10 equiv), **1** or **2** (5 equiv), DMF, rt, 18 h, 42% **7**, 51% **8**, 73% **11**, 68% **12**. (ii) CF_3COOH/CH_2Cl_2 (8/2), rt, 2 h, quantitative yields **9–14**.

the protecting groups (Boc), the expected compounds **13** and **14** were obtained in quantitative yields.

Protoporphyrin IX polyamine derivatives **15, 16** were synthesised as described in a previous paper.[7]

2.2. Mass characterization (MALDI)

Mass spectrometry of all porphyrin polyamine derivatives was performed using the MALDI-TOF (matrix-assisted laser desorption ionization-time-of-flight) technique. Most of the compounds studied gave one main peak (protonated molecule MH^+, no fragments). Nevertheless, compounds bearing polyamine units with protective groups (Boc), gave additional signals.

2.3. ^{1}H NMR characterization

^{1}H NMR (400.13 MHz) was used for the characterization of compounds **3–8** and **11–12** dissolved in $CDCl_3$ and compounds **9, 10** and **13, 14** dissolved in CD_3OD. The detailed resonance assignments were based on integration and selective homonuclear decoupling and 2D homonuclear COSY experiments. NMR spectra of these compounds are governed by the symmetry properties of the molecules (number of polyamine units attached to the macrocycle). For *meso* tetrapolyamine arylporphyrins **7** and **8**, resonances of the eight equivalent pyrrolic protons appear as a single peak at 8.81 ppm (**7**) and 8.78 ppm, respectively (**8**). In contrast, each pair of adjacent pyrrolic protons of *trans*-dipolyamine arylporphyrins **5**, **6**, **9** and **10** gave distinct signals split into two doublets.

2.4. UV–vis spectra

Porphyrins synthesized in this work show typical electronic spectra in CH_2Cl_2, with a Soret band near 420 nm and four less intense visible Q bands (I, II, III and IV) (Table 1). In aqueous solutions, electronic spectra of compounds with free amino groups (**9**, **10** and **13–16**) showed quite large differences. All the unprotected compounds presented a blue shift of the Soret band as compared to corresponding protected compounds. *Cis* polyamine porphyrin derivatives (**15** and **16**) showed an additional broadening of the Soret band; this behaviour could be attributed to face-to-face stacking.[12]

Table 1
UV–vis spectra (λ_{nm} ($\varepsilon \times 10^{-3}$, $cm^{-1}\,mol^{-1}\,L$)) of porphyrin derivatives in various solvents[a]

Compounds	Soret	Visible bands (Q)
5(**a**)	419 (392.4)	515 (18.3), 549 (7.5), 591 (5.4), 648 (4.7)
6(**b**)	414 (144.8)	512 (6.0), 546 (2.7), 589 (1.7), 645 (1.3)
7(**a**)	419 (307.1)	515 (13.5), 550 (6.4), 590 (4.0), 646 (3.0)
8(**a**)	419 (266.8.)	516 (14.1), 551 (6.9), 591 (4.4), 646 (3.6)
9(**b**)	416 (230.6)	513 (8.7), 549 (7.2), 593 (3.6), 645 (1.8)
9(**c**)[b]	412 (142.4)	519 (7.3), 556 (4.3), 585 (2.7), 646 (1.7)
10(**b**)	415 (204.7)	513 (8.6), 547 (4.7), 588 (2.7), 645 (1.5)
10(**c**)[b]	414 (182.7)	517 (5.3), 554 (3.8), 582 (2.6), 636 (1.5)
11(**a**)	419 (306.3)	515 (13.5), 549 (5.4), 591 (4.1), 646 (2.7)
12(**a**)	419 (338.2)	515 (14.8), 549 (6.1), 590 (4.4), 646 (3.0)
13(**b**)	415 (330.5)	513 (2.6), 546 (6.1), 589 (4.3), 645 (2.7)
13(**c**)[b]	414 (285.0)	517 (11.9), 533 (5.2), 581 (4.2), 635 (2.6)
14(**b**)	415 (318.8)	513 (12.7), 545 (2.1), 589 (3.7), 646 (2.7)
14(**c**)[b]	414 (390.9)	517 (14.5), 552 (6.5), 581 (5.4), 634 (3.2)
15(**b**)	403 (27.0)	503 (2.5), 538 (2.1), 575 (1.3), 629 (0.9)
15(**c**)	399 (59.9)	505 (5.5), 540 (4.5), 569 (2.8), 626 (1.0)
16(**b**)	402 (29.7)	503 (2.6), 538 (2.1), 575 (1.4), 629 (0.9)
16(**c**)	402 (95.3)	505 (7.8), 541 (6.8), 571 (4.4), 624 (2.5)

[a] Solvents as follows: (a) CH_2Cl_2, (b) MeOH, (c) H_2O.
[b] Soret band shows a shoulder.

3. Photophysical studies

3.1. Photostability of porphyrin polyamine derivatives

In order to establish if unprotected porphyrin polyamine derivatives **9**, **10**, **13**, **14, 15** and **16** could undergo photobleaching, we performed photostability studies in the same conditions of irradiation used for DNA photocleavage experiments (fluence rate 2.5 mW/cm^2). Results (Table 2) display the relative residual absorbance of samples in function of irradiation time.[13] Under these conditions, all compounds show a high photostability with a virtual absence of photobleaching after 1 h of irradiation.

3.2. Singlet oxygen production

Singlet oxygen production was evaluated during irradiation of porphyrin polyamine conjugates by monitoring the decay of 1,

Table 2
Behaviour of the photosensitizers after irradiation (white light, fluence 2.5 mW/cm^2) for different periods of time

Photosensitizers	Irradiation time (min)				
	0	15	30	45	60
9	100	100	99	98	97
10	100	100	100	99	97
13	100	100	100	100	99
14	100	100	99	99	98
15	100	100	99	98	97
16	100	100	100	100	97

Absorbance was measured at the maximum of the Soret band. Results represent residual absorbance (%) in function of irradiation time.

3-diphenylisobenzofuran (DPBF) whose reaction with singlet oxygen leads to the formation of an endoperoxide with a drop in absorbance at 415 nm.[14] Solutions of porphyrins **9, 10** and **13**–**16** containing DPBF were irradiated and absorbance was measured at 3-min intervals. As shown in Figure 1, absorbance decline of DPBF is enhanced in the presence of the photosensitizers. So, these results show that all polyamine porphyrin derivatives studied produce singlet oxygen and that no significant difference in production rate was observed among these compounds.

3.3. Partition coefficients

Lipophilicity has proven an important molecular descriptor that often is well-correlated with the bioactivity of drugs; log *P*, which reflects the equilibrium partitioning of a molecule between a non polar and a polar phase, such as the 1-octanol/water system.[15] In this work, we have determined log *P* of porphyrin–tetrapolyamine derivatives **9**, **10**, (*trans*) porphyrin dipolyamines **13** and **14** and protoporphyrin dipolyamines **15** and **16** as log ($[porphyrin]_{1\text{-octanol}}$/$[porphyrin]_{water}$).

3.4. Interaction with calf thymus DNA

Results presented in Figure 2, show UV–vis polyamine–porphyrins spectral changes following serial addition of calf thymus DNA.

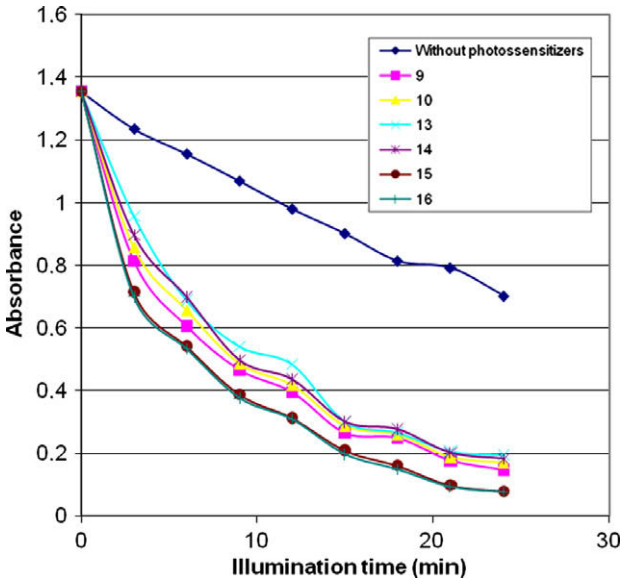


Figure 1. Absorbance decay of DPBF in absence or in presence of photosensitizers **9**, **10**, **13**, **14** and **15**, **16**. Solutions of DPBF (6×10^{-5} M) and photosensitizers (10^{-6} M) in DMF/H_2O (9/1) were irradiated at room temperature with two white bulbs (30 W each) giving a light fluence of 10 mW/cm^2. Absorbance at 415 nm was monitored in function of time.

These spectra illustrate the influence of peripheral substituents on binding modes.[16] These data show that increasing DNA concentration leads to marked hypochromicity and red shift of the Soret band of tetrapolyamine porphyrin derivatives **9**, **10** and, more obviously, of protoporphyrin IX polyamine derivatives **15**, **16**,. Each one of these four spectral changes is characterized by a single isosbestic point, typical of an equilibrium between free and DNA-bound porphyrin.[17] These strong spectral modifications suggest that these compounds interact with DNA by a combination of outside binding and intercalation.[18] On the other hand, Soret bands of *trans*-dipolyamine porphyrin derivatives **13** and **14** show a small red shift and a slight hypochromicity corresponding to a weak interaction with DNA (outside binding). These results suggest that the positions of the positive charges (polyamine functions) are very important for determining binding affinity.[19]

4. DNA photocleavage

4.1. Photocleavage of supercoiled pBR322 plasmid

Comparison of the DNA cleaving efficiencies of polyamine–porphyrin conjugates is shown in Figure 3. Agarose gel electrophoresis separates supercoiled DNA (I) from the relaxed circular form (II) as shown in lane 2. The linearized form (III) obtained after EcoRI digestion displays an intermediate migration (lane 1). As shown in lane 3, no cleavage was observed when DNA alone was subjected to illumination up to 2 h. Moreover, control experiments indicated that no cleavage of DNA did occur if compounds were mixed with DNA without irradiation (lanes 4 and 10). Assays were conducted with *cis* di-substituted **15**, **16** (a), *trans*-di-substituted **13**, **14** (b) and tetra-substituted porphyrins **9**, **10** (c). Lanes 5 to 9 and 11 to 15 show the effects of increasing irradiation time for spermidine and spermine conjugates, respectively. The cleaving ability is revealed by a progressive disappearance of the supercoiled plasmid (I), and a concomitant increase of the relaxed circular form (II) accounting for at least one break in one of the two DNA strands; after prolonged irradiation the linear form shows up, as a consequence of breaks affecting both DNA strands. Results show that the cleaving ability depends on the relative position of polyamine substitutions since *cis* di-substituted porphyrins exhibit cleavage ability (Fig. 3a) contrary to *trans*-di-substituted porphyrins (Fig. 3b). The latter were tested at a higher DNA concentration (100 μM) and DNA-photosensitizer mixtures were irradiated during 2 h with no apparent change in DNA electrophoresis pattern, thus confirming the inactivity of *trans*-di-substituted derivatives (data not shown).

Furthermore, results reported in Figure 2 show that protoporphyrin IX dispermine derivative **16** was more efficient than the analogous compound substituted with spermidine (compound **15**, Fig. 3a); in fact, the lowest active concentrations of compounds **16** and **15** were 1 μM and 2.5 μM, respectively (data not shown). This discrepancy between spermine and spermidine derivatives was also observed with tetra-substituted compounds although the latter proved less efficient than the corresponding *cis*-disubstituted ones (Fig. 3c).

4.2. DNA photocleavage inhibition

DNA photocleavage inhibition assays were conducted with protoporphyrin IX polyamine derivatives **15** and **16**, the most efficient compounds of this series. Results are reported in Figure 4. The reported results account for a strong photocleavage inhibition in presence of 0.1 M sodium azide and, to a lesser extent, in the presence of 0.1 M mannitol (lanes 8 and 11). As sodium azide is a well known $^1O_2$ quencher and mannitol is a hydroxyl radical scavenger,

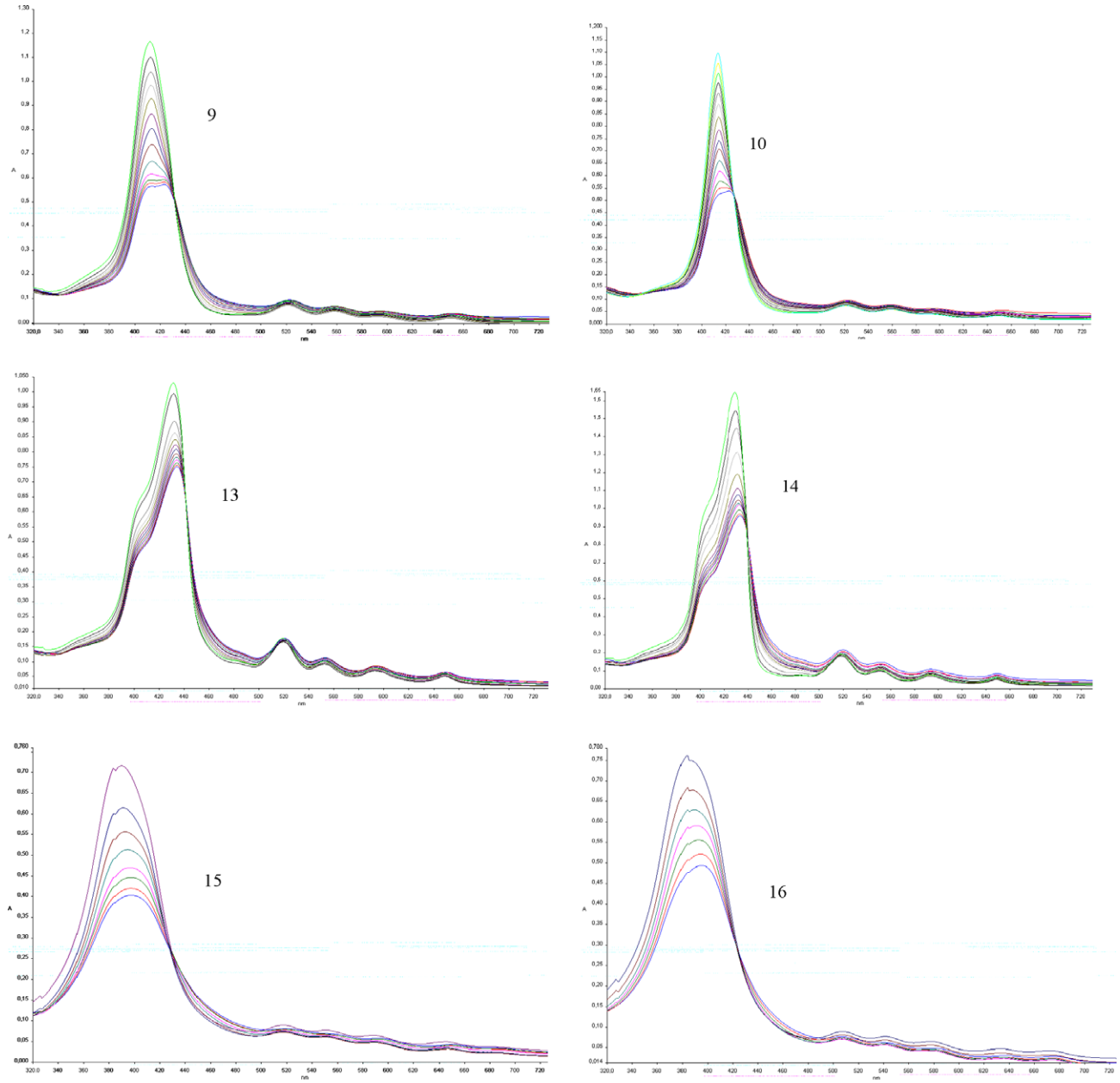


Figure 2. UV–vis absorbance of 4×10^{-6} M porphyrins **9**, **10**, **13**, **14**, **15** and **16** with increasing concentrations of calf thymus DNA. Base pair concentration: 0 (upper curves) to 1.2×10^{-5} M (lower curves). Spectra were recorded in phosphate buffer (pH 7.4).

our results indicate that singlet oxygen is the major species responsible for the cleavage of DNA.

5. Discussion

In this study, we have investigated the synthesis of 6 new polyamine photosensitizers which differ by the nature, number and position of the polyamine substituents on the macrocycle. The choice of the number and position of polyamine units of the porphyrin rings was dictated by the necessity to modulate the hydrophilic/lipophilic balance of the molecule which seems to be a very important factor to favour cell transport and uptake.[20] The six compounds examined do not have the same solubility in aqueous medium. So, *trans* porphyrin polyamine derivatives **13**, **14** and protoporphyrin IX polyamine derivatives **15**, **16** are slightly soluble in water with log *P* near 0 which seems to be characteristic of amphiphilic molecules. The remaining compounds **9**, **10** were shown to have relatively low partition coefficient (log $P < -2$) which indicates their very high water solubility (Table 3). Many factors could affect the photocleavage of DNA as the production of singlet oxygen or the number and position of polyamine units on the porphyrin rings. First of all, we have studied singlet oxygen production by measuring the disappearance of 1,3-diphenylisobenzofuran (DPBF) in presence of these porphyrins. It was found that the six compounds studied produce singlet oxygen and no significant difference was observed among these porphyrins (Fig. 1). So, this finding implied that photocleavage abilities of these photosensitizers were controlled by their DNA binding modes. In the present work DNA binding study, realized by spectrophotometric titration of porphyrins in the presence of increasing concentrations of calf thymus DNA, show various degrees of DNA interaction according to the number and position of positive charges (polyamine units) on the porphyrin macrocycle (Fig. 2). All synthesized compounds were able to bind DNA but photosensitizers bearing four and two

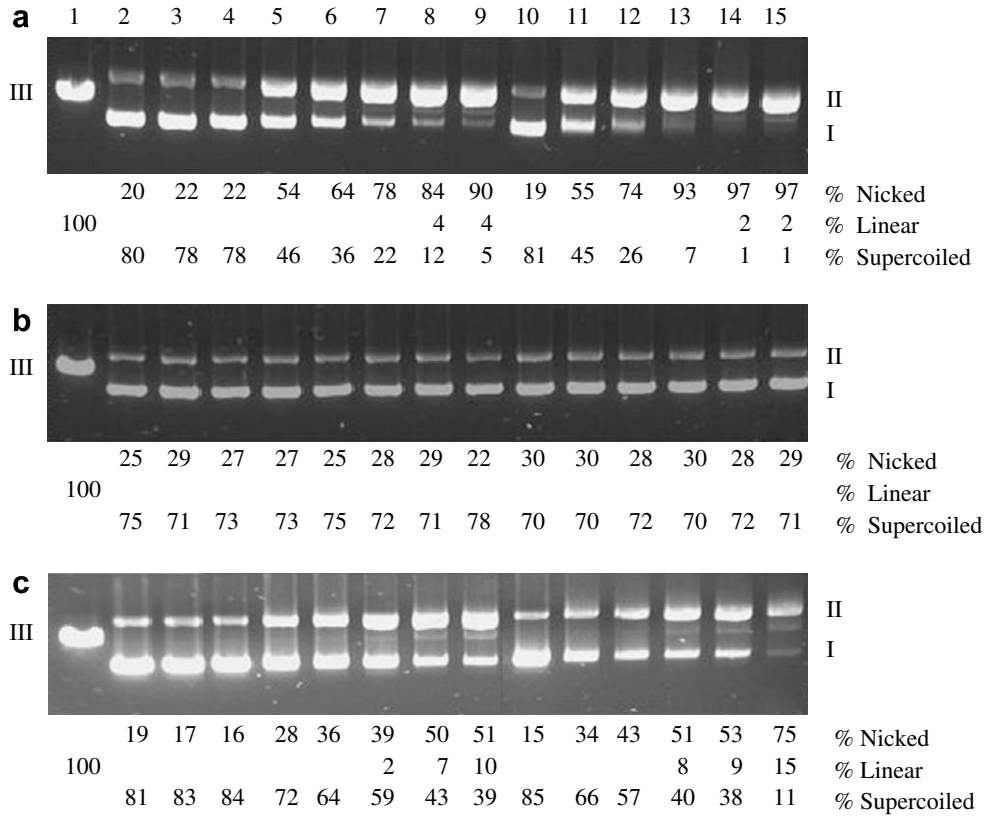


Figure 3. Cleavage of supercoiled pBR322 by *cis*-disubstituted **15** and **16**(a), *trans*-disubstituted **13** and **14**(b) and tetra-substituted **9** and **10**(c) porphyrins. Reaction mixtures (10 μL) containing 0.25 μg of plasmid DNA ± porphyrin (10 μM) were exposed (or not) to white light irradiation (2.5 mW/cm^2). Lane 1: linearized plasmid III (EcoRI digestion); lane 2: DNA alone (I: supercoiled and II: relaxed circular plasmid); lane 3: DNA irradiated 120 min; lanes 4–9: DNA + spermidine compounds **15**(a), **13**(b) and **9**(c); lane 4: no irradiation, lane 5: 15 min, 6: 30 min, 7: 60 min, 8: 90 min and 9: 120 min irradiation; lanes 10–15: DNA + spermine compounds **16**(a), **14**(b) and **10**(c); lane 10: no irradiation, lane 11: 15 min, 12: 30 min, 13: 60 min, 14: 90 min and 15: 120 min irradiation.

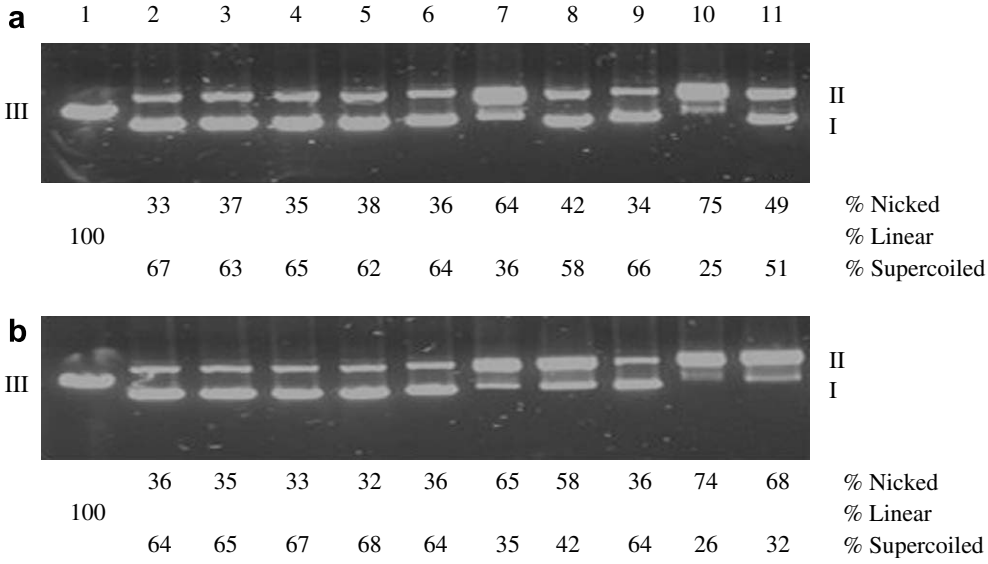


Figure 4. Effect of sodium azide and mannitol on pBR322 photocleavage. Reaction mixtures (10 μL) containing 0.25 μg of plasmid DNA ± cisdisubstituted protoporphyrine polyamine derivatives **15** (spermidine) and **16** (spermine) (10 μM) were exposed or not to irradiation (2.5 mW/cm^2) in the presence of 0.1 M sodium azide (a) or 0.1 M mannitol (b) for 60 min. Lane 1: linearized plasmid III (EcoRI digestion); lane 2: DNA alone (I: supercoiled and II: relaxed circular plasmid); lane 3: DNA irradiated 60 min; lane 4: DNA + NaN_3/mannitol; lane 5: DNA + NaN_3/mannitol with irradiation, lane 6: DNA + compound **15**; lane 7: DNA + compound **15** with irradiation; lane 8: DNA + compound **15** + NaN_3/mannitol with irradiation; lane 9: DNA + compound **16**, lane 10: DNA + compound **16** with irradiation, lane 11: DNA + compound **16** + NaN_3/mannitol with irradiation.

(*cis)* polyamine units (respectively **9**, **10**, and **15**, **16**) displayed larger hypochromicities as well as stronger red shifts of the Soret bands in comparison to *trans*-di-substituted polyamine porphyrin conjugates **13**, **14**. This result suggests that the number of positive

charges on the same side of the macrocycle is essential for the binding to DNA.

In the aim to confirm these results, we observed the ability of these cationic porphyrins to photocleave DNA. We studied the conversion of supercoiled pBR322 (form I) into relaxed form (II) and/or linearized form (III). Figure 3 shows the photocleavage abilities of compounds **15** and **16**, bearing two polyamines on the same side of the macrocycle, whereas compounds **13** and **14**, characterized by two polyamines attached on opposite sides of the macrocycle proved consistently inactive in this respect. These data correlate with the different kinds of interaction with calf thymus DNA previously observed by UV–vis spectrophotometry for *trans*-di-polyamine porphyrins derivatives **13**, **14** and protoporphyrin IX polyamine derivatives **15**, **16**. Obviously, polyamine units must be attached on the same side of the photosensitizer, in order to increase the number of electrostatic interactions between the positive charges (amine functions) of porphyrins and the negative charges of phosphate groups. In this context, even if tetra-substituted porphyrins possess four polyamine units, this feature does not increase photocleavage efficiencies. The steric effect of compounds **9** and **10** may be responsible for the lower DNA photocleavage abilities as compared to compounds **15** and **16**. Concerning the nature of the polyamine substitution on porphyrin, our results show that spermine derivatives are more efficient than spermidine ones (Fig. 2), suggesting the importance of the number of amine groups and consequently the number of positive charges on the porphyrin ring (Scheme 1). Indeed, the increase of Coulombic forces between positive and negative charges favours the interaction between photosensitizers and DNA.[21] The implication of amine groups is confirmed by assays conducted with precursor of compounds **16** bearing two Boc-protected spermines. These protected compounds did not exhibit any DNA photocleavage ability (data not shown).

DNA photocleavage reported here can be due to two kinds of reactions after photoactivation of the photosensitizers. One of them involves the generation of free radicals (type I photochemical reaction) and the second one, the production of singlet oxygen, O_2 ($1\Delta g$) (type II photochemical reaction); the latter is frequently reported as the main species responsible for DNA cleavage.[22] Since all these four polyamine porphyrins produce singlet oxygen, it was of interest to assess the implication of this species in the DNA photocleavage of pBR322. Results show that the conversion of supercoiled plasmid into the relaxed form brought by DNA strand cleavage(s) is essentially inhibited by sodium azide and to a lesser extent by mannitol (Fig. 4). As NaN_3 is a well known $^1O_2$ quencher and mannitol is a hydroxyl radical scavenger, our results confirm the importance of type II reaction in DNA photocleavage. Owing to the short life time (<0.4 μs) and the short diffusion distance (<0.01 μm) of singlet oxygen,[23] the DNA binding ability appears as a prerequisite for DNA photocleavage induced by type II reaction).

6. Conclusion

A series of new polyamine porphyrin-conjugates has been designed, synthesized and characterized by ^1H NMR, UV–vis and MALDI. All compounds are efficient singlet oxygen generators (DPBF photooxidation) and show a high photostability. UV–vis spectral changes indicate that *cis*-dipolyamine protoporphyrin IX derivatives **15**, **16** strongly bind to DNA, a result underscored by the DNA photocleavage efficiency of these compounds. Our results also suggest that the number, together with the positions of the polyamines on the macrocycle play important roles in determining their binding to DNA and therefore their photocleavage abilities. Consequently, these two cis-disubstituted photosensitizers may have potential cell death ability by inducing irreversible alterations of genomic DNA. To confirm if compounds **15** and **16** will be good candidates for using in PDT we have to assess their efficiency on tumour cells.

Table 3
Partition coefficients of porphyrin–polyamine conjugates (determinations were repeated three times)

Compound	**9**	**10**	**13**	**14**	**15**	**16**
Log *P*	−2.02	−2.83	0.11	0.07	−0.12	−0.51

7. Experimental

7.1. Material and instrumentation

All solvents and reagents were purchased from Aldrich, Prolabo or Acros. Pyrrole and dimethylformamide were distilled over CaH_2 under reduced pressure immediately before use. Methylene chloride and chloroform were distilled over P_2O_5, then CaH_2. Analytical thin-layer chromatography (TLC) was performed on silica gel (Merck, $60F_{254}$). Merck precoated plates (silica gel 60, 2 mm) were used for preparative thin-layer chromatography. Column chromatography was carried out with silica gel (60 ACC, 15–40 μm, Merck). UV–vis spectra were obtained in 1 or 0.1 cm quartz cells and recorded by using a Perkin–Elmer LS-5B spectrophotometer. ^1H NMR spectra were recorded in CD_3OD, $CDCl_3$ or DMSO-d_6 with tetramethylsilane as an internal standard. The chemical shifts are given in ppm and coupling constants in Hz. MALDI mass spectra were obtained on a Voyager Elite (Framingham MA–USA) time-of-flight mass spectrometer by the Laboratoire de Chimie Structurale Organique et Biologique, Université Pierre et Marie Curie, Paris.

7.2. Syntheses

meso-(Mesityl)dipyrromethane, **4**, 5,10,15,20-tetra(4-carboxyphenyl) porphyrin **3** and protoporphyrin IX polyamine derivatives 15, 16 were synthesised as described in a previous paper.[7,9]

7.2.2. Synthesis of porphyrins

7.2.2.1. 5,15-Bis(4-methylesterphenyl)10,20-bis(mesityl)porphyrin (5). *meso*-(Mesityl)dipyrromethane **4** (132 mg, 0.5 mmol, 1 equiv) and methyl-4-formylbenzoate (82 mg, 0.5 mmol, 1 equiv) were dissolved in 48 mL of CH_2Cl_2. Then, 66 μL of TFA (0.3 mmol, 1.8 equiv) in CH_2Cl_2 (2 mL) were slowly added and the mixture was stirred at room temperature during 30 min. *p*-Chloranil (123 mg, 0.5 mmol, 1 equiv) was added and the mixture was stirred during 1 h. Then, the mixture was cooled and the solvent evaporated to dryness; crude product was purified by column (alumina) and thin-layer chromatography (CH_2Cl_2). Compound **5** (60 mg) was obtained (yield 30%).

R_f = 0.54 (CH_2Cl_2). UV–vis (CH_2Cl_2): λ_{max} (nm, $\varepsilon \times 10^{-3}$ mol^{-1} L cm^{-1}) = 419 (392.4), 515 (18.3), 549 (7.5), 591 (5.4), 648 (4.7). ^1H NMR (δ 400.13 MHz, $CDCl_3$, 25 °C) −2.63 (s, 2H, N**H** pyrrole), 1.83 (s, 12H, C**H$_3$**-*o*-mesityl), 2.62 (s, 6H, C**H$_3$**-*p*-Mesityl), 4.10 (s, 6H, COOC**H$_3$**), 7.29 (s, 4H, **H**-*m*-mesityl), 8.31 (d, *J* = 8.2 Hz, 4H, **H$_{2,6}$** aryl), 8.42 (d, *J* = 8.2 Hz, 4H, **H$_{3,5}$** aryl), 8.71 (d, *J* = 4.7 Hz, 4H, **H$_\beta$** pyrrole), 8.74 (d, *J* = 4.7 Hz, 4H, **H$_\beta$** pyrrole). SM (MALDI): *m*/*z* 815.25 $[M+H]^+$.

7.2.2.2. 5,15-Bis(4-carboxyphenyl)10,20-bis(mesityl)porphyrin (6). Porphyrin **5** (110 mg, 0.14 mmol, 1 equiv) was dissolved in DMF (5 mL) and KOH (2 mL, 1 M in ethanol) was added. The mixture was stirred under reflux for 45 min. After cooling, solvent was evaporated under vacuum and the residue was dissolved in MeOH.

The solution was neutralized by addition of acidic resin and then, compound **6** precipitated. After filtration, 100 mg of porphyrin **6** were obtained (94%).

R_f = 0.48 (CH_2Cl_2/EtOH: 94/6 + 1% CH_3COOH). UV–vis (MeOH): λ_{max} (nm, $\varepsilon \times 10^{-3}$ mol^{-1} L cm^{-1}) = 414 (144.8), 512 (6.0), 546 (2.7), 589 (1.7), 645 (1.3). ^1H NMR (δ 400.13 MHz, CD_3OD, 25 °C) 1.82 (s, 12H, C**H$_3$**-*o*-mesityl), 2.61 (s, 6H, C**H$_3$**-*p* mesityl), 7.31 (s, 4H, **H**-*m* mesityl), 8.21 (d, *J* = 8.1 Hz, 4H, **H$_{2,6}$** aryl), 8.37 (d, *J* = 8.1 Hz, 4H, **H$_{3,5}$** aryl), 8.68 (br s, 4H, **H$_\beta$** pyrrole), 8.77 (br s, 4H, **H$_\beta$** pyrrole). SM (MALDI): *m*/*z* 787.36 $[M+H]^+$.

7.2.3. General procedure for the synthesis of porphyrins bearing spermine or spermidine units

N^4-(4-Aminobutyl)-N^1,N^8-bis-*t*-butoxycarbonylpermidine **1** (4.4 equiv or 5 equiv), or N^4-(4-aminobutyl)-N^1,N^8,N^{12}-tris-*t*-butoxycarbonylspermine **2** (4.4 equiv or 5 equiv) were dissolved in DMF. A solution of carboxy-porphyrins **3** or **6** (1 equiv), and *N*,*N*′-dicyclohexylcarbodiimide (DCC) (1.1 equiv or 10 equiv) in dry DMF (15 mL) was added. After addition of 1-hydroxybenzotriazole (HOBt) (1.1 equiv or 10 equiv), the mixture was kept at room temperature, in the dark, under argon, for 18 h. DMF was evaporated under vacuum and the crude product was dissolved in chloroform. The organic layer was washed with water (2 × 50 mL), dried on $MgSO_4$ and then evaporated to afford, after purification by thin-layer chromatography the pure product.

7.2.3.1. 5,10,15,20-Tetra(N^1,N^8-bis-*tert*-butoxycarbonylspermidine(N^4-(4-aminobutyl)4-amidophenyl))porphyrin (7). 5,10, 15, 20-Tetra(4-carboxyphenyl) porphyrin **3** (523 mg, 0.7 mmol), DCC (0.6 g, 2.9 mmol), HOBt (393 mg, 2.9 mmol) and compound **1** (1.21 g, 2.9 mmol) gave 667 mg of **7** (42%).

R_f = 0.42 ($CHCl_3$/EtOH: 7/3 + 2% Et_3N). UV–vis (CH_2Cl_2): λ_{max} (nm, $\varepsilon \times 10^{-3}$ mol^{-1} L cm^{-1}) = 419 (307.1), 515 (13.5), 550 (6.4), 590 (4.0), 646 (3.0); ^1H NMR (δ $CDCl_3$, 400.13 MHz), δ = −2,81 (br s, 2H, NH pyr.), 1.43 (s, 72H, C**H$_3$** Boc), 1.53 (m, 16H, N–CH_2–(C**H$_2$**)$_2$–CH_2–NHBoc), 1.67 (br t, 16H, $J_{H,H}$ = 6.3 Hz, Porph–CO–NH–$(CH_2)_2$–C**H$_2$**–CH_2–N–CH_2–C**H$_2$**–CH_2–NHBoc), 1.81 (m, 8H, Porph–CO–NH–CH_2–C**H$_2$**–$(CH_2)_2$–N), 2.45(br t, *J* = 6.2 Hz 24H, Porph–CONH–$(CH_2)_3$–C**H$_2$**–N–C**H$_2$**–$(CH_2)_3$NHBoc and N–C**H$_2$**–$(CH_2)_2$ NHBoc), 3.24 (m, 8H, N–$(CH_2)_3$–C**H$_2$**–N), 3.16 (m, 8H, N–$(CH_2)_2$–C**H$_2$**–NHBoc), 3.64 (m, 8H, Porph–O–C**H$_2$**–), 4.88 (br s, 4H, N**H**Boc), 5.35 (br s, 4H, N**H**Boc), 7.01 (br s, 4H, Porph-CON**H**–) 8.21 (m, 8H, $H_{2,6}$ Aryl), 8.25 (d, 8H, *J* = 7.1 Hz $H_{3,5}$ aryl), 8.81 (s, 8H, H_β pyr.).MS (MALDI) *m*/*z*: 2387.57 $[M+H]^+$.

7.2.3.2. 5,10,15,20-Tetra(N^1,N^8,N^{12}-tris-*tert*-butoxycarbonylspermine(N^4-(4-aminobutyl)4-amidophenyl))porphyrin (8). 5,10, 15,20-tetra(4-carboxyphenyl) porphyrin **3** (523 mg, 0.7 mmol), DCC (0.6 g, 2.9 mmol), HOBt (393 mg, 2.9 mmol) and compound **2** (1.21 g, 2.9 mmol) gave 1.0 g of **8** (51%).

R_f = 0.62 ($CHCl_3$/EtOH: 7/3 + 2% Et_3N). UV–vis (CH_2Cl_2): λ_{max} (nm, $\varepsilon \times 10^{-3}$ mol^{-1} L cm^{-1}) = 419 (266.8), 516 (14.1), 551 (6.9), 591 (4.4), 646 (3.6); ^1H NMR (δ $CDCl_3$, 400.13 MHz), δ = −2.83 (br s, 2H, NH pyr.), 1.44 (s, 108H, C**H$_3$** Boc), 1.63 (m, 32H, –N–CH_2–(C**H$_2$**)$_2$–CH_2Nboc–CH_2–C**H$_2$**–CH_2NHBoc and N–CH_2–C**H$_2$**–CH_2NHBoc), 1.86 (m, 16H, Porph–CONH–CH_2–(C**H$_2$**)$_2$–CH_2N), 2.83 (m, 32H, Porph–CONH$(CH_2)_3$–C**H$_2$**–N–C**H$_2$**–$(CH_2)_3$NBoc–C**H$_2$**–$(CH_2)_2$NHBoc and N–C**H$_2$**–$(CH_2)_2$NHBoc), 3.08–3.24 (m, 24H, N–$(CH_2)_3$–C**H$_2$**–NHBoc–$(CH_2)_2$–C**H$_2$**–NHBoc and N–$(CH_2)_2$–C**H$_2$**–NHBoc). 3.72 (m, 8H, Porph–CONH–C**H$_2$**– $(CH_2)_3$–N), 4.82 (br s, 8H, N**H**Boc), 5.33 (br s, 4H, Porph–CON**H**–), 8.22 (br s, 16H, $H_{2,6}$ and $H_{3,5}$ aryl), 8.78 (br s, 8H, H_β pyr.). MS (MALDI) *m*/*z*: 3014.92 $[M+H]^+$.

7.2.3.3. 5,15-Bis(N^1,N^8 -bis-*tert*-butoxycarbonylspermidine(N^4-(4-aminobutyl)4-amidophenyl))10,20-bis (mesityl)porphyrin (11). Porphyrin **6** (113 mg, 0.14 mmol), DCC (296 mg, 1.4 mmol), HOBt (194 mg, 1.4 mmol) and compound **1** (299 mg, 7.2 mmol) gave 166 mg of **11** (73%).

R_f = 0.48 ($CHCl_3$/EtOH: 7/3 + 2% Et_3N). UV–vis (CH_2Cl_2): λ_{max} (nm, $\varepsilon \times 10^{-3}$ mol^{-1} L cm^{-1}) = 419 (306.3), 515 (13.5), 549 (5.4), 591 (4.1), 646 (2.7); ^1H NMR (δ $CDCl_3$, 400.13 MHz), δ = −2,65 (br s, 2H, NH pyr), 1.42 (s, 36H, C**H$_3$** Boc), 1.83 (s, 12H, CH_3-*o*-mesityl), 2.63 (s, 6H, CH_3-*p*-mesityl), 1.55–1.94 (m, 20H, Porph–CO–NH–CH_2–(C**H$_2$**)$_2$–CH_2–N–CH_2–(C**H$_2$**)$_2$–CH_2–NHBoc and N–CH_2–C**H$_2$**–CH_2–NHBoc), 3.22 (m, 12H, Porph–CONH–$(CH_2)_3$–C**H$_2$**–N–C**H$_2$**–$(CH_2)_3$NHBoc and N–C**H$_2$**–$(CH_2)_2$NHBoc), 3.38 (m, 4H, N–$(CH_2)_2$–C**H$_2$**–NHBoc or N–$(CH_2)_3$–C**H$_2$**–NHBoc), 3.48 (m, 4H, N–$(CH_2)_3$–C**H$_2$**–NHBoc or N–$(CH_2)_2$–C**H$_2$**–NHBoc), 3.68 (m, 4H, Porph–NHCO–C**H$_2$**–), 4.26 (br s, 2H, N**H**Boc), 4.87 (br s, 2H, N**H**Boc), 6.06 (br s, 2H, Porph-CON**H**-) 7.28 (s, 4H, $H_{2,6}$ mesityl), 8.24 (br s, 4H, $H_{2,6}$ aryl), 8.27 (d, 4H, *J* = 7.1 Hz $H_{3,5}$ aryl), 8.69 (d, *J* = 4.7 Hz, 4H, H_β pyr.). 8.73 (d, *J* = 4.7 Hz, 4H, H_β pyr.). MS (MALDI) *m*/*z*: 1584.13 $[M+H]^+$.

7.2.3.4. 5,15-Bis(N^1,N^8,N^{12}-tris-*tert*-butoxycarbonylspermine (N^4 -(4-aminobutyl)4-amidophenyl))10,20-bis (mesityl)porphyrin (12). Porphyrin **6** (100 mg, 0.13 mmol), DCC (262 mg, 1.3 mmol), HOBt (172 mg, 1.3 mmol) and compound **2** (365 mg, 0.63 mmol) gave 163 mg of **12** (68%).

R_f = 0.56 ($CHCl_3$/EtOH: 7/3 + 2% Et_3N). UV–vis (CH_2Cl_2): λ_{max} (nm, $\varepsilon \times 10^{-3}$ mol^{-1} L cm^{-1}) = 419 (338.2), 515 (14.8), 549 (6.1), 590 (4.4), 646 (3.0); ^1H NMR (δ $CDCl_3$, 400.13 MHz), δ = −2.64 (br s, 2H, NH pyr.), 1.44 (s, 54H, C**H$_3$** Boc), 1.83 (s, 12H, CH_3-*o*-mesityl), 2.63 (s, 6H, CH_3-*p*-mesityl), 1.52 (m, 8H, Porph–CONH$(CH_2)_4$–N–CH_2–(C**H$_2$**)$_2$–CH_2NBoc–), 1.63–1.70 (m, 16H, Porph–CONH–CH_2–(C**H$_2$**)$_2$–CH_2N– and NBoc–CH_2–C**H$_2$**–CH_2NHBoc and N–CH_2–C**H$_2$**–CH_2NHBoc), 2.58 (m, 32H, Porph–CONH$(CH_2)_3$–C**H$_2$**–N–C**H$_2$**–$(CH_2)_3$NBoc–C**H$_2$**–$(CH_2)_2$NHBoc and N–C**H$_2$**–$(CH_2)_2$NHBoc), 3.22–3.28 (m, 24H, N–$(CH_2)_3$–C**H$_2$**–NHBoc–$(CH_2)_2$–C**H$_2$**–NHBoc and N–$(CH_2)_2$–C**H$_2$**–NHBoc). 3.64 (m, 4H, Porph–CONH–C**H$_2$**–$(CH_2)_3$–N), 4.92 (br s, 4H, N**H**Boc), 5.29 (br s, 2H, Porph–CON**H**–), 7.28 (s, 4H, $H_{2,6}$ mesityl), 8.22 (br s, 4H, $H_{2,6}$ aryl), 8.28 (d, *J* = 7.5Hz, 4H, $H_{3,5}$ aryl), 8.70 (d, *J* = 4.6 Hz, 4H, H_β pyr.), 8.74 (d, *J* = 4.7 Hz, 4H, H_β pyr.). MS (MALDI) *m*/*z*: 1898.16 $[M+H]^+$ (calcd 1898.56).

7.2.4. General procedure for removal of Boc protective groups

The protecting groups (Boc) were removed with standard method in high yields with TFA in CH_2Cl_2 at room temperature (2 h).

7.2.4.1. 5,10,15,20-Tetra(spermidine(N^4-(4-aminobutyl)4-amidophenyl))porphyrin (9). R_f = 0.80 (CH_3CN/H_2O: 7/3 + 1% TFA). UV–vis (CH_3OH): λ_{max} (nm, $\varepsilon \times 10^{-3}$ mol^{-1} L cm^{-1}) = 416 (230.6), 513 (8.7), 549 (7.2), 593 (3.6), 645 (1.8); ^1H NMR (δ CD_3OD, 400.13 MHz), 1.27 (m, 40H, Porph–CO–CH_2–(C**H$_2$**)$_2$–CH_2–N–CH_2–(C**H$_2$**)$_2$–CH_2–NH_2 and N–CH_2–C**H$_2$**–CH_2–NH_2); 2.95 (m, 24H, Porph–CO–$(CH_2)_3$–C**H$_2$**–N–C**H$_2$**–$(CH_2)_3$–NH_2 and N–C**H$_2$**–$(CH_2)_2$–NH_2), 3.04 (br t, *J* = 6.4 Hz, 16H, N–$(CH_2)_3$–C**H$_2$**–NH_2 and N–$(CH_2)_2$–C**H$_2$**–NH_2), 3.11 (t, *J* = 7.2 Hz, 8H, Porph–CO–C**H$_2$**–$(CH_2)_3$NH), 8.30 (d, *J* = 8.3 Hz, 8H, $H_{2,6}$ aryl), 8.33 (d, *J* = 8.3 Hz 8H, $H_{3,5}$ aryl), 8.84 (br s, 8H, H*β*-pyrrole), MS (MALDI) *m*/*z*: 1584.08 $[M+H]^+$.

7.2.4.2. 5,10,15,20-Tetra(spermine(N^4-(4-aminobutyl)4-amidophenyl))porphyrin (10). R_f = 0.71 (CH_3CN/H_2O: 7/3 + 1% TFA). UV–vis (CH_3OH): λ_{max} (nm, $\varepsilon \times 10^{-3}$ mol^{-1} L cm^{-1}) = 415 (204.7), 513 (8.6), 547(4.7), 588 (2.7), 645 (1.5). ^1H NMR (CD_3OD, 400.13 MHz): δ 1.91 (m, 32H, $(CH_2)_2NCH_2CH_2CH_2CH_2NH$–$CH_2CH_2CH_2NH_2$ and $(CH_2)_2NCH_2CH_2CH_2CH_2NH_2$); 2.02 (m, 16H, Porph–CONH–CH_2–(C**H$_2$**)$_2$–CH_2–N), 2.20 (m, 16H, –NH–$(CH_2)_2$–C**H$_2$**–NH_2 and –N–$(CH_2)_2$–C**H$_2$**–NH_2) 3.23 (m, 40H, Porph–CONH–$(CH_2)_3$–C**H$_2$**–N–C**H$_2$**–$(CH_2)_2$–C**H$_2$**–NH–C**H$_2$**–$(CH_2)_2$–NH_2 and –N–C**H$_2$**–$(CH_2)_2$–NH_2), 3.67 (br t, *J* = 6.3 Hz, 8H, Porph–CONH–C**H$_2$**–

$(CH_2)_3$–N), 8.29 (d, J = 8.0 Hz, 8H, $H_{2,6}$ aryl), 8.31 (d, J = 8.0 Hz, 8H, $H_{3,5}$ aryl), 8.82 (br s, 8H, Hβ-pyrrole); MS (MALDI) m/z: 1812.28 $[M+H]^+$.

7.2.4.3. 5,15-Bis(spermidine(N^4-(4-aminobutyl)4-amidophenyl))10,20-bis(mesityl)porphyrin (13). R_f = 0.63 (CH_3CN/H_2O: 7/3 + 1% TFA). UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 415 (330.5); 513 (2.6); 546 (6.1); 589 (4.3); 645 (2.7). ^{1}H NMR (CD_3OD, 400.13 MHz): δ 1.49–1.71 (m, 10H, CO–NH–CH_2–(C**H$_2$**)$_2$–CH_2–N and N–CH_2–(C**H$_2$**)$_2$–CH_2–NH_2 and N–CH_2–C**H$_2$**–CH_2–NH_2) 1.75 (s, 12H, CH_3-*o*-mesityl), 2.51–2.77 (m, 10H, CO–NH–CH_2–$(CH_2)_2$–C**H$_2$**–N and N–C**H$_2$**–$(CH_2)_2$–C**H$_2$**–NH_2 and N–C**H$_2$**–CH_2–C**H$_2$**–NH_2), 2.57 (s, 6H, CH_3-*p*-mesityl), 7.25 (s, 2H, $H_{3,5}$ mesityl), 8.20 (d, J = 7.2 Hz, 4H, $H_{2,6}$ aryl), 8.27 (d, J = 7.9 Hz, 4H, $H_{3,5}$ aryl), 8.67 (br s, 4H, Hβ-pyrrole), 8.78 (br s, 4H, Hβ-pyrrole), MS (MALDI) m/z: 1183.85 $[M+H]^+$.

7.2.4.4. 5,15-Bis(spermine(N^4-(4-aminobutyl)4-amidophenyl))-10,20-bis (mesityl)porphyrin (14). R_f = 0.73 (CH_3CN/H_2O: 7/3 + 1% TFA). UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 415 (318.8); 513 (12.7); 545 (2.1); 589 (3.7); 646 (2.7). ^{1}H NMR (CD_3OD, 400.13 MHz): δ 1.25–1.55 (m, 12H, CO–NH–CH_2–(C**H$_2$**)$_2$–CH_2–N and N–CH_2–(C**H$_2$**)$_2$–CH_2–NH–CH_2–C**H$_2$**–CH_2–NH_2 and N–CH_2–C**H$_2$**–CH_2–NH_2), 1.74 (s, 12H, CH_3-*o*-mesityl), 2.29–2.91 (m, 14H, CO–NH–CH_2–$(CH_2)_2$–C**H$_2$**–N and N–C**H$_2$**–$(CH_2)_2$–C**H$_2$**–NH–C**H$_2$**–CH_2–C**H$_2$**–NH_2 and N–C**H$_2$**–CH_2–C**H$_2$**–NH_2), 2.55 (s, 6H, CH_3-*p*-mesityl), 7.23 (s, 2H, $H_{3,5}$ mesityl), 8.22 (br s, 4H, $H_{2,6}$ aryl), 8.26 (br s, 4H, $H_{3,5}$ aryl), 8.66 (br s, 4H, Hβ-pyrrole), 8.77 (br s, 4H, Hβ-pyrrole), MS (MALDI) m/z: 1297.93 $[M+H]^+$.

7.3. Partition coefficient measurements

1-Octanol/water partition coefficients were determined at 25 °C using equal volumes of water (3 mL) and 1-octanol (3 mL). Typically a 300 μM solution of each dye (**9**, **10** and **13–16**) was vortexed and centrifuged, 100 μL aliquots of aqueous and organic phases were separately diluted, each one into 2 mL MeOH and the final dye concentrations were determined by absorption spectroscopy.[24]

7.4. Singlet oxygen production

Photosensitizers (10^{-6} M) and 1,3-diphenylbenzofuran (6×10^{-5} M) were dissolved in DMF/H_2O (9/1). The mixtures were illuminated at room temperature during 30 min with two white bulbs (30 W each) giving a fluence of 10 mW/cm^2. Absorbance decay of DPBF at 415 nm was measured at time intervals of 3 min.

7.5. DNA interaction assay

Interactions between cationic porphyrins and calf thymus DNA were tested by UV–vis spectroscopy[8d] Calf thymus DNA (Invitrogen) was dissolved in PBS (pH 7.4). Initially, visible absorption spectrum of each polyamine–porphyrin conjugate was measured at a concentration of 4×10^{-6} M at Soret maximum absorption. Calf thymus DNA was added (base pair concentrations from 1.2×10^{-6} M to 1.2×10^{-5} M), base pair/porphyrin ratio running from 0.2 to 3.

7.6. DNA photocleavage assay

The photocleavage abilities of porphyrins **9**, **10** and **13–16** were assayed on plasmid DNA (supercoiled pBR322) and products of illumination were analyzed by agarose gel electrophoresis. In a total volume of 10 μL, individual reactions containing 0.25 μg of plasmid where performed at 25 °C in buffer (3 mM Tris–HCl, 0.3 mM EDTA, pH 8.0)[25] and the samples (±10 μM porphyrin) were exposed to a 18 W lamp which placed 4 cm away for various periods of time (15, 30, 60, 90 and 120 min). For each assay, control experiments were conducted on pBR322 without porphyrin and/or without irradiation. Cleavage products were then electrophoresed in a 0.7% non-denaturing agarose gel, stained with SYBRsafe (Invitrogen) and visualized on a transilluminator set at 312 nm.

7.7. DNA photocleavage inhibition

Individual reactions containing 0.25 μg of plasmid DNA pBR322 and 10 μM of protoporphyrin IX polyamine conjugates **15** and **16** were prepared in buffer (3 mM Tris–HCl, 0.3 mM EDTA, pH 8.0) and irradiated 60 min in the presence of 100 mM sodium azide or 100 mM mannitol.[26] Additional control experiments were realized on pBR322 without porphyrin in the presence of sodium azide or mannitol and with or without irradiation. DNA photocleavage inhibition was assessed using agarose gel electrophoresis as described above.

Acknowledgements

We thank the Conseil Régional du Limousin' for financial support and for a fellowship attributed to one of us (G. G.). The authors thank Dr. Michel Guilloton for help in writing the manuscript.

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