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Polyamine conjugates of *meso*-tritolylporphyrin and protoporphyrin IX: Potential agents for photodynamic therapy of cancers

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Abstract—An efficient five-step synthesis method was developed to obtain tritolylporphyrin and protoporphyrin IX polyamine conjugates. These compounds were composed of either one polyamine unit (spermidine or spermine) covalently tethered to monocarboxyphenyl tritolylporphyrin or two molecules of polyamines borne by protoporphyrin IX. In each compound, an aliphatic spacer arm is linked to the N^4 polyamine position. Photocytotoxicity of these new compounds was evaluated against K562 human chronic myelogenous leukemia cells and compared to Photofrin II®; protoporphyrin IX polyamine conjugates exhibited much stronger photocytocicity than Photofrin II® and were shown to readily induce necrosis in treated cells.

1. Introduction

Photodynamic therapy (PDT) is an established modality for treatment of neoplasic diseases. PDT involves selective accumulation of photosensitizer by cancer cells and in situ photoactivation of the photosensitizer by visible light, leading to the destruction of treated cells.[1] Two types of photoreaction mechanisms are invoked to explain photosensitizer action: light-activated photosensitizers in its triplet state can generate free radicals by electron or proton transfer (type I photochemical reactions) or singlet oxygen ($^1O_2$) is produced by energy transfer (type II reactions). Singlet oxygen seems to be the major mediator of photochemical cell damage,[2] yet the mechanism of action is not well understood. Photofrin II® is an efficient first-generation photosensitizer approved for PDT but it suffers from several drawbacks such as a lack of selectivity toward tumor cells and a persistent photosensitization of the skin. At present, considerable efforts are being devoted to the development of new PDT agents. Among them, *meta*-tetrahydroxyphenylchlorin (*m*-THPC), also known by trade name Foscan®, received regulatory approval, in 2002 in the European Union for palliative treatment of head and neck cancer.[3] Benzoporphyrin derivative monoacid ring A (BPD-MA) trade named Visudyne® (Verteporfin for injection) is used in Phase III clinical trial for basal cell carcinoma (BCC) and Mono-L-aspartylchlorin e6 (MACE) is being developed in Japan, under the acronym NPe6, for the treatment of endobronchial lung tumours.[4]

Vectored or targeted drugs, which have enhanced affinity for cancer cells, would be an important advance in cancer therapy. To this end, we have devised a synthetic route to obtain porphyrin derivatives designed for selective uptake by tumor cells and for binding DNA. Polyamines such as spermine, spermidine or putrescine, required for optimal growth and replication of various cell types, are present in high concentrations in rapidly proliferating cells.[5] Cancer cells, whose polyamine requirements exceed biosynthetic capabilities, use a polyamine transport system (PAT) to fulfill their needs. This system displays at the same time a strong affinity and a low specificity for polyamines. Therefore, polyamine transport system can afford selective accumulation of polyamine analogs in neoplastic tissues and presents a very attractive anticancer chemotherapeutic strategy.[6,7] Literature study has shown that one can

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utilize polyamine conjugation to convey cytotoxic drugs (acridine-carborane, chloroambucil, nitroimidazole) into rapidly growing cells.[8] On the other hand, polyamines are known to interact with the phosphate moiety of nucleic acids by charge interaction and by hydrogen bonding. So, in their cationic form, these compounds constitute an interesting class of DNA binding molecules.[7a,9,10]

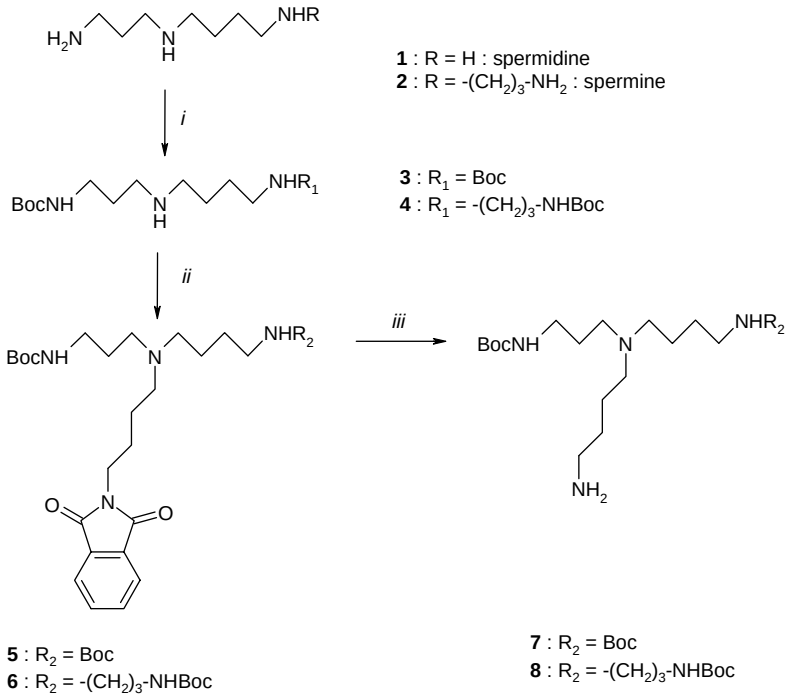
In connection with our research program on porphyrin and their use in PDT, it occurred to us that porphyrin-based sensitizers in combination with an intracellular recognition element might acquire 'dual action' capabilities.[11,12] To this end, we thought to generate a suitable hybrid porphyrin with polyamine moieties. In this paper, we report the synthesis of porphyrin derivatives **16**–**19** bearing spermine and spermidine linked by spacer arm on the macrocycle. As porphyrins moiety, we used either a synthetic porphyrin as tritolylporphyrin which bore one polyamine unit (spermidine or spermine) **16**, **17** or protoporphyrin IX bearing two units of polyamines **18**, **19**. In all cases, spermine and spermidine analogs **7**, **8** have been attached to the porphyrin core by amide linkages.

2. Results

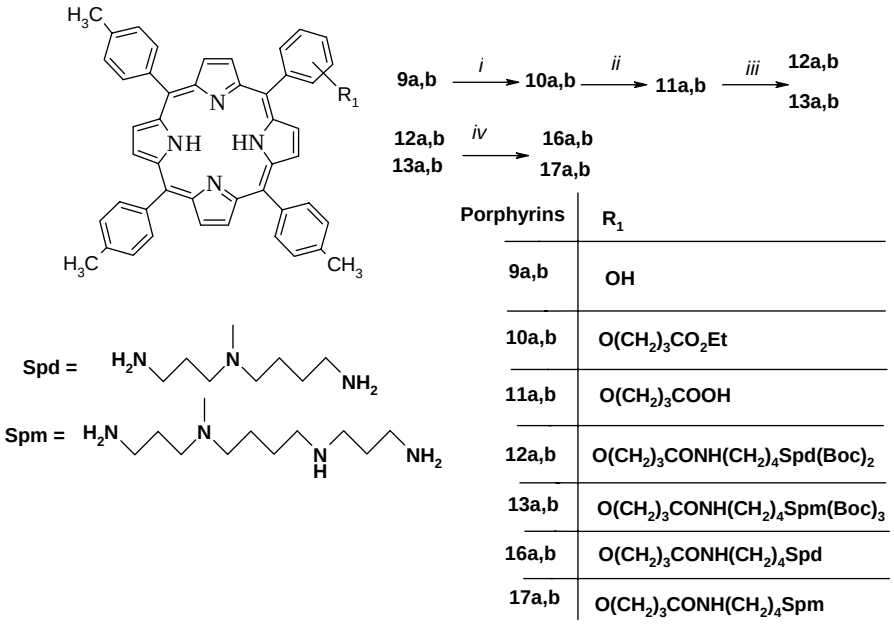
2.1. Synthesis of porphyrins

Different approaches to the selective protection of polyamines have been reported.[13] We chose Boc-protective group, which can be selectively removed by trifluoroacetic acid (TFA). Thus, spermidine **1** and spermine **2** (Scheme 1) have been protected using 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile (Boc-ON) in THF as described in our preliminary work,[11c] to obtain in one step, respectively, spermidineBoc_2 **3** (83%) and spermineBoc_3 **4** (72%).[14] Then, compounds **3** and **4** were monoalkylated with *N*-(4-bromobutyl)phthalimide in acetonitrile in the presence of potassium carbonate and the yields obtained for resulting compounds **5** and **6** were 96% and 95%, respectively. In each case, spermidine and spermine were conjugated at the N^4 position via an aliphatic carbon tether to the phtalimide nucleus. This central attachment was predicted by the findings by Poter in 1982,[13] wherein spermidine could be derivatized at the central N^4 position and could be taken up by the polyamine transporter.[15] The N^4 alkylation step was designed to maintain the basicity of N^4 nitrogen, which was also shown to be critical for uptake. Hydrazinolysis of compounds **5** and **6** gave the respective amines **7** (95%) and **8** (86%).

meso-Monohydroxyphenyltritolylporphyrins **9a**,**b** (Scheme 2) were synthesized by Little's standard method (Adler modified method), condensation of pyrrole (4 equiv) with *para*-tolylaldehyde (3 equiv) and *para*-or *ortho*-hydroxybenzaldehyde (1 equiv) in propionic acid giving porphyrins *para* **9a** in 6% yield and porphyrins *ortho* **9b** in 5% yield. These compounds were converted into the derivatives **10a**,**b** by treatment with ethyl 4-bromobutyrate (10 equiv) with K_2CO_3 (20 equiv) in dry DMF at room temperature for 18 h. Purification of the resulting products on TLC gave 90% and 85% yields for **10a** and **b**, respectively. The carboxy-function-



Scheme 1. Reagents and conditions: (i) with **1**, Boc-ON (2 equiv), THF, 0 °C, 18 h, 83% ; with **2**, Boc-ON (3 equiv), THF, 0 °C, 18 h, 72%; (ii) *N*-(4-bromobutyl)phthalimide (1.25 equiv), K_2CO_3 (5 equiv), CH_3CN, rt,18 h, 96% **5**, 95% **6**; (iii) hydrazine monohydrate (50 equiv), THF/EtOH 80:20, 90 °C 5 h then 50 °C 18 h, 95% **7**, 86% **8**.



Porphyrins	R_1
9a,b	OH
10a,b	$O(CH_2)_3CO_2Et$
11a,b	$O(CH_2)_3COOH$
12a,b	$O(CH_2)_3CONH(CH_2)_4Spd(Boc)_2$
13a,b	$O(CH_2)_3CONH(CH_2)_4Spm(Boc)_3$
16a,b	$O(CH_2)_3CONH(CH_2)_4Spd$
17a,b	$O(CH_2)_3CONH(CH_2)_4Spm$

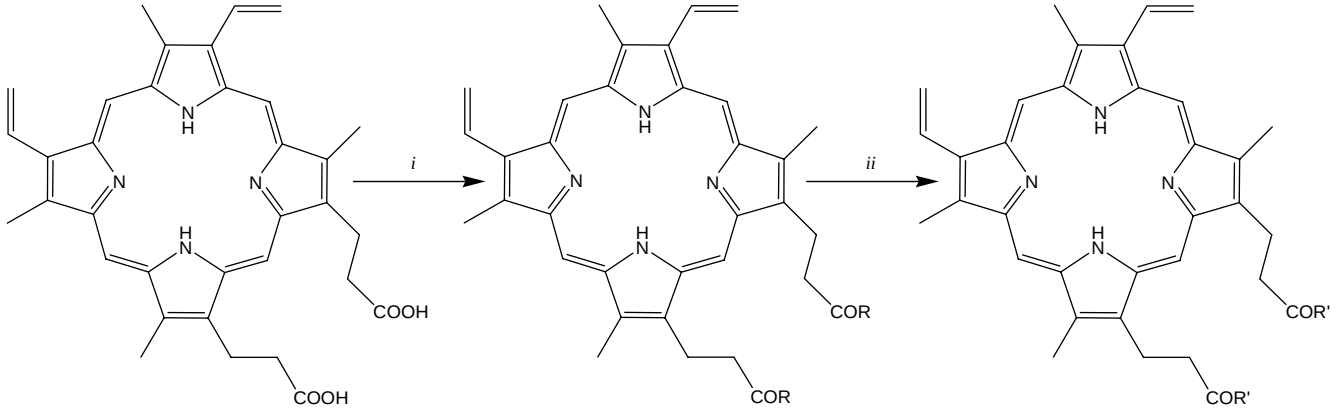
a and b refer to *para* and *ortho* position of R_1 group respectively

Scheme 2. Reaction conditions: (i) $Br(CH_2)_3CO_2Et/K_2CO_3$/DMF, 18 h, rt; (ii)KOH/EtOH/DMF 2 h, reflux; (iii) polyamine **7** or **8** (1.1 equiv), DCC (1.1 equiv), HOBt (1.1 equiv), DMF, rt, 72 h 80% **12a**,**b**, 85%, **13a**,**b**; (iv) CF_3COOH/CH_2Cl_2 (1:1), rt, 2 h, quantitatively yields **16a**,**b** and **17a**,**b**.

alized porphyrins **11a**,**b** were obtained in excellent yields by saponification of compounds **10a** and **b** with KOH/EtOH (1 M in DMF) at reflux for 2 h.

The synthesis of polyamine porphyrins **12a**,**b** and **13a**,**b** was carried out by reaction of carboxy-porphyrins **11a**,**b** 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 the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in dimethylformamide. Finally, the protecting groups (Boc) were removed by standard methods in high yields with trifluoroacetic acid in dichloromethane at room temperature (2 h). Finally, the attempted porphyrins were obtained in a nearly quantitative yield.

Under the same conditions, protoporphyrin IX (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 the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in DMF. After purification by TLC (eluent: CH_2Cl_2/EtOH 80:20 + 1% Et_3N), protected polyamine porphyrin conjugates **14** and **15** were obtained in 78% and 84% yields, respectively. After cleavage of protecting groups (Boc), the expected compounds **18** and **19** were obtained in quantitative yields.



Protoporphyrin IX

14 : R=NH-$(CH_2)_4$-spermidineBoc_2
15 : R=NH-$(CH_2)_4$-spermineBoc_3

18 : R'=NH-$(CH_2)_4$-spermidine
19 : R'=NH-$(CH_2)_4$-spermine

Scheme 3. Reagents and conditions: (i) Protoporphyrin IX (1 equiv), polyamine **7** or **8** (2.2 equiv), DCC (2.2 equiv), HOBt (2.2 equiv), DMF, rt, 72 h 80% **14**, 85%, **15**; (ii) CF_3COOH/CH_2Cl_2 (1:1), rt, 2 h, quantitatively yields **18** and **19**.

2.2. Mass characterization

Mass spectrometry of all porphyrin polyamine derivatives was performed using the matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) technique. Most of the studied compounds gave one main peak (protonated molecule MH^+, no fragments). Nevertheless, compounds bearing polyamine units with protective groups (Boc) gave additional signals. The MALDI-TOF mass spectrum of compound **13a** (Fig. 1) clearly indicated the presence of the protonated porphyrin derivatives ($M+H^+$) along with metastable fragment ions that corresponded to the loss of one, two, and three protective groups, respectively.

2.3. ^{1}H NMR characterization

^{1}H NMR spectra recorded at 400.13 MHz were used for characterization of **9**–**13** in $CDCl_3$ or $CDCl_3/CD_3OD$ (9/1) for **16**–**17**. The detailed resonance assignments are based on integration and selective homonuclear decoupling and 2D homonuclear COSY experiments. The spectra of these compounds are governed by the symmetry properties of the molecule and by the orientation of the spacer arm in **10**–**11**, polyamines bearing Boc units in **12**–**13** (Fig. 2), and polyamines with free amino functions in **14**–**15**. Porphyrin derivatives that possess one *meso* phenyl group with *ortho* substitution (**9b**, **10b**, **11b**, **12b**, and **13b**), displayed an obvious change in the chemical shift of the phenyl ring.[11b] Furthermore, compounds (**12b**, **13b**) which are characterized by *ortho* spermidine and spermine substitutions, are subjected to considerable changes in the ^{1}H NMR chemical shift and/or in figure relative to most of the nuclei. Thus, for example, all the protons of spacer arm and polyamine (spermine or spermidine) experience a strong shielding from −0.12 ppm (CH_3 Boc) to −1.43 ppm (H_γ, spacer arm). These nuclei are obviously located well within the range of the shielding current above the porphyrin macrocycle.

^{1}H NMR spectra of protoporphyrin IX polyamine conjugates were realized in $CDCl_3$ for **14**–**15** and in CD_3OD for **18**–**19**. We have observed all chemical shifts and figures relative to most of the nuclei of protoporphyrin IX and polyamine units.

2.4. UV–vis absorption

The *meso*-phenylporphyrin conjugates **9**–**11** synthesized in this work show typical electronic spectra, with a Soret band near 420 nm and four less intense Q bands with an *etio* outline. Polyamine- protected *meso*porphyrin conjugates **12**–**13** were characterized by basically identical *etio* spectra with very little difference in extinction coefficients. On the other hand, the unprotected counterparts

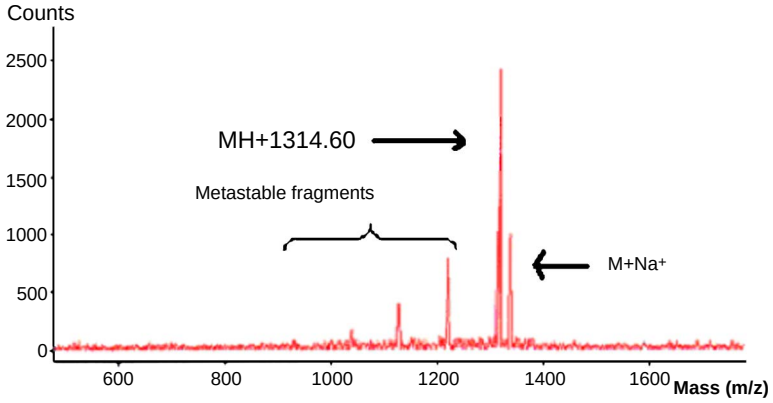


Figure 1. Example of MALDI mass spectra for compound **13a**.

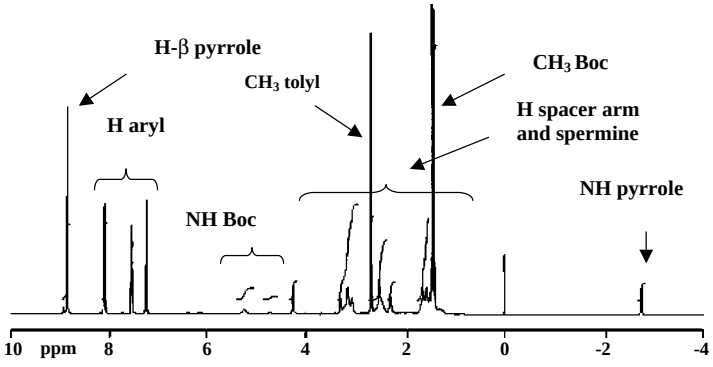


Figure 2. ^{1}H NMR spectrum in $CDCl_3$ of compound **13a**.

Table 1. UV–vis spectra [λ_{nm} ($\varepsilon \times 10^{-3}$, cm^{-1} mol^{-1} L)] of protoporphyrin-polyamine conjugates in organic solvents (CH_2Cl_2, MeOH) and water

Derivatives	Soret	Q IV	Q III	Q II	Q I
14[a]	407 (113.9)	504 (8.8)	541 (8.4)	575 (5.3)	629 (3.2)
15[a]	406 (133.5)	504 (10.5)	540 (9.3)	574 (6.6)	629 (3.7)
18[b]	403 (27.0)	503 (2.5)	538 (2.1)	575 (1.3)	629 (0.9)
19[b]	402 (29.7)	503 (2.6)	538 (2.1)	575 (1.4)	629 (0.9)
18[c]	399 (59.9)	505 (5.5)	540 (4.5)	569 (2.8)	626 (1.0)
19[c]	402 (95.3)	505 (7.8)	541 (6.8)	571 (4.4)	624 (2.5)

The solvents used are as follows:
[a] CH_2Cl_2.
[b] MeOH.
[c] Water.

16-17 in methanol solution displayed a difference in Soret band height with respect to substitution positions (Experimental): the extinction coefficients of *para* substituted compounds **16a**, **17a** were lower than the extinction coefficients of *ortho* substituted compounds **16b**, **17b** although electronic configuration should not be modified.

With regard to protoporphyrin-polyamine conjugates **14**, **15** and **18**, **19**, their UV–vis spectra are typical of protoporphyrin IX, consisting of the intense Soret band around 408 nm and weaker Q bands in the 500–650 nm interval. Nevertheless, in water, their molar absorption coefficients decrease as shown in Table 1 and the Soret band is blueshifted and broadened. These features strongly suggest an association of protoporphyrin polyamine conjugates in aqueous solutions.[16]

2.5. Partition coefficients

In medicinal chemistry, lipophilicity has proven to be an important molecular descriptor that often is well correlated with the bioactivity of drugs. Lipophilicity is indicated, for example, by the logarithm of a partition coefficient, log *P*, which reflects the equilibrium partitioning of a molecule between a nonpolar and a polar phase, such as the 1-octanol/water system. In this work, we have determined log *P* of tritolylporphyrin-polyamine **16**, **17** and protoporphyrin-polyamine derivatives **18**, **19** as $\log([Porphyrin]_{1\text{-octanol}}/[Porphyrin]_{water})$ which indicates that compounds **16**, **17** are more lipophilic than **18**, **19** (Table 2). Determinations were repeated three times.

2.6. Singlet oxygen production

In order to determine the photosensitizing properties of porphyrins **16**, **17**, **18**, and **19**, trapping reactions of $^1O_2$ with ergosterol acetate were carried out.[17] Reference experiments with eosin, rose bengal or hematoporphyrin (HP), known singlet oxygen producers, gave ergosterol acetate epidioxide with nearly quantitative yields. In the same experimental conditions, our porphyrins had the same efficiency for $^1O_2$ production than HP.

Table 2. Partition coefficient of tritolylporphyrin and protoporphyrin-polyamine conjugates

Derivatives	**16a**	**16b**	**17a**	**17b**	**18**	**19**
Log *P*	>3[a]	>3[a]	>3[a]	>3[a]	−0.12	− 0.51

[a] Nearly insoluble in water.

2.7. Biological tests

The results presented in Figure 3 show that porphyrin polyamine conjugates **18** and **19** at 2×10^{-6} M display a very strong photocytotoxicity, even for the shortest irradiation time. These effects were much stronger than with Photofrin II® used at an equivalent ponderal concentration. Lowering porphyrin conjugate concentrations to 2×10^{-7} M resulted in a drop in toxicity so that their effects were quite similar to those of Photofrin II® whatever the irradiation time, if the dead cells were counted at once after irradiation.

Although irradiation in the presence of Photofrin® resulted in an increase in dead cells after an additional 24 h incubation in the dark (Fig. 3E, solid bars), such an effect was not seen when polyamine conjugates **18** or **19** were used (Figs. 3C and D, solid bars). This discrepancy could indicate a difference in the death pathways induced by either Photofrin® or polyamine conjugates.

Biological assays were also conducted with tritolylporphyrin monopolyamine conjugates **16**, **17** at 2×10^{-6} M (Fig. 4). We have observed a less important photocytotoxicity, especially for short irradiation times; it is worth noticing that photoactivity displayed by *ortho* compounds **16b**, **17b** (Figs. 4B and D) was always significantly stronger than their *para* counterparts **16a**, **17a** (Figs. 4A and C).

Annexin V-FITC/propidium iodide (PI) fluorescent cell staining was used to check cell death induced by polyamine porphyrins **18** and **19**. Results presented in Fig. 5 show that the massive death recorded after 30 min irradiation in the presence of 2×10^{-6} M porphyrin polyamine **19** was probably the consequence of a rapid necrotic death: more than 80% of the treated cells became nonviable (PI permeable cells) although they did not display the strong FITC fluorescence characteristic of apoptotic cells (Fig. 5A). Irradiation in the presence of the same compounds at 2×10^{-7} M resulted in a much lower nonviable cell count, again without any consistent increase in FITC fluorescence (Fig. 5B). Experiments conducted with porphyrin polyamine **18**

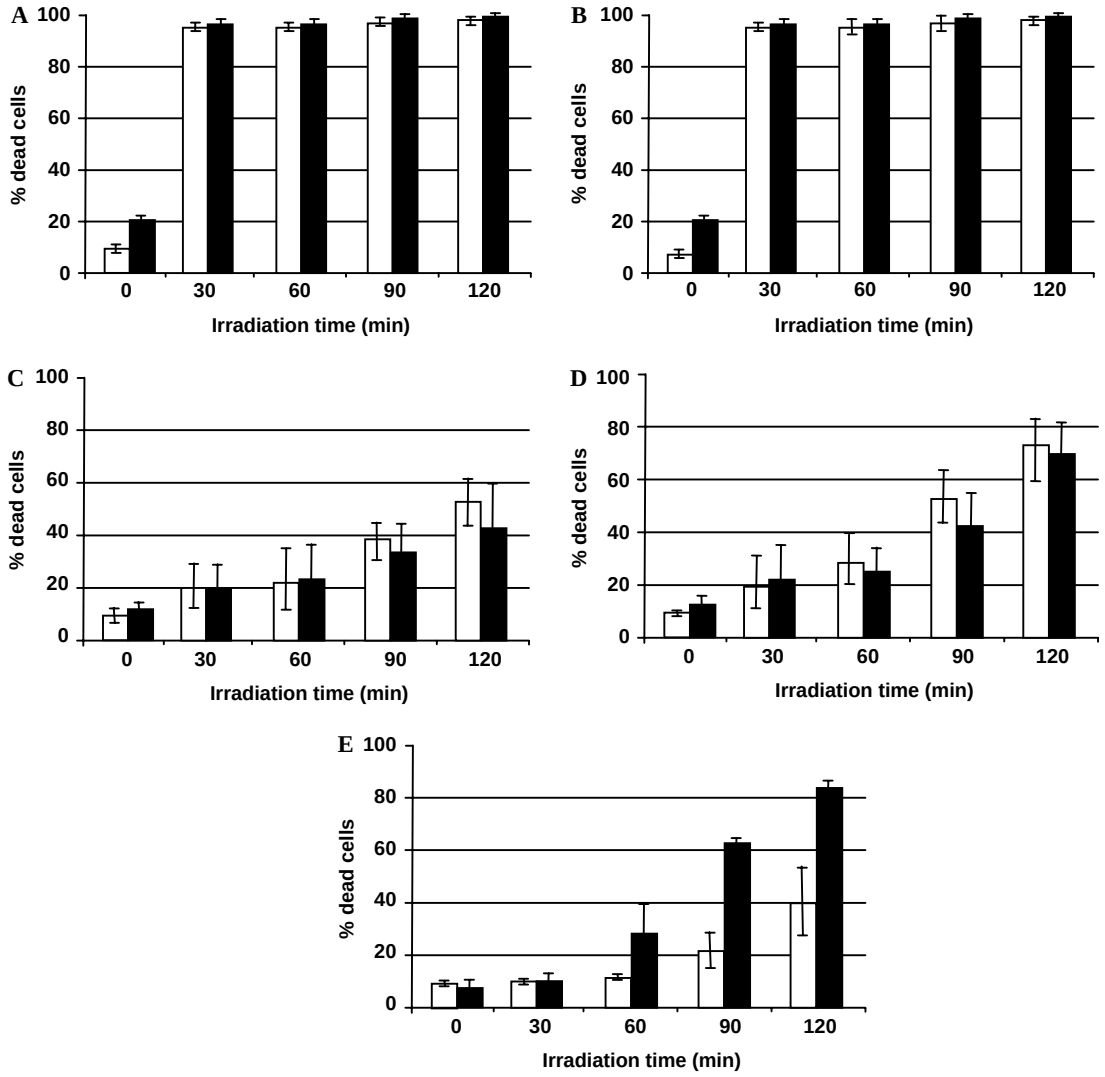


Figure 3. Cell irradiation and analysis were conducted as described in Experimental. Cells were incubated with porphyrin polyamine conjugates **18** ((A) 2×10^{-6} M, (C) 2×10^{-7} M) or **19** ((B) 2×10^{-6} M, (D) 2×10^{-7} M), or 1.25 μg/mL Photofrin II® (E). Dead cell count was estimated by flow cytometry just after irradiation (void bars) or after a further 24 h incubation in the dark (solid bars). Error bars are based upon standard deviations.

led to virtually identical results (not shown). The same irradiation in the presence of 1.25 μg mL^{-1} Photofrin® (Fig. 5D) resulted in a slight increase in FITC-stained viable cell population. These results are consistent with photocytotoxicity tests presented in Figure 3, since irradiation in the presence of Photofrin® is followed by an increase in dead cell count within the next 24 h, contrary to irradiation in presence of porphyrin polyamines **18** and **19**.

3. Discussion

In this study, we have investigated the synthesis of six new polyamine-photosensitizers which differ by the nature, number, and position on macrocycle of polyamine units. We have tried to correlate these different structures with cell killing efficiency. The choice of the number and position of polyamine units of the porphyrin rings was didacted by the necessity to modulate the hydrophilic/lipophilic balance of the molecule which seems to be a very important factor to increase transport and uptake in cells.[18] The six drugs examined do not have the same solubility in aqueous medium. So, only protoporphyrin IX polyamine derivatives **18**, **19** are soluble in water with $\log P$ near 0 which seems to be characteristic of an amphiphilic molecule. The other compounds **16a**,**b** and **17a**,**b** were shown to have a relatively high partition coefficient ($\log P > 3$) which indicates their very low water solubility. Based on the results of the loss of cell viability, we observed that none of the compounds studies exhibited cytotoxicity in the dark. Moreover, after photoactivation with white light, polyamine porphyrin conjugates **18** and **19** at 2×10^{-6} M displayed a very strong photocytotoxicity, higher than that of Photofrin®, even for the shortest irradiation time (95% of death cells after 30 min of irradiation). Thus, the efficacy of the photoactivity is influenced by the hydrophophilic/lipophilic character of the compounds; the presence of two polyamine units on the same side of the macrocycle (**18**–**19**) increases in parallel amphiphilic character and phototoxicity of these molecules.

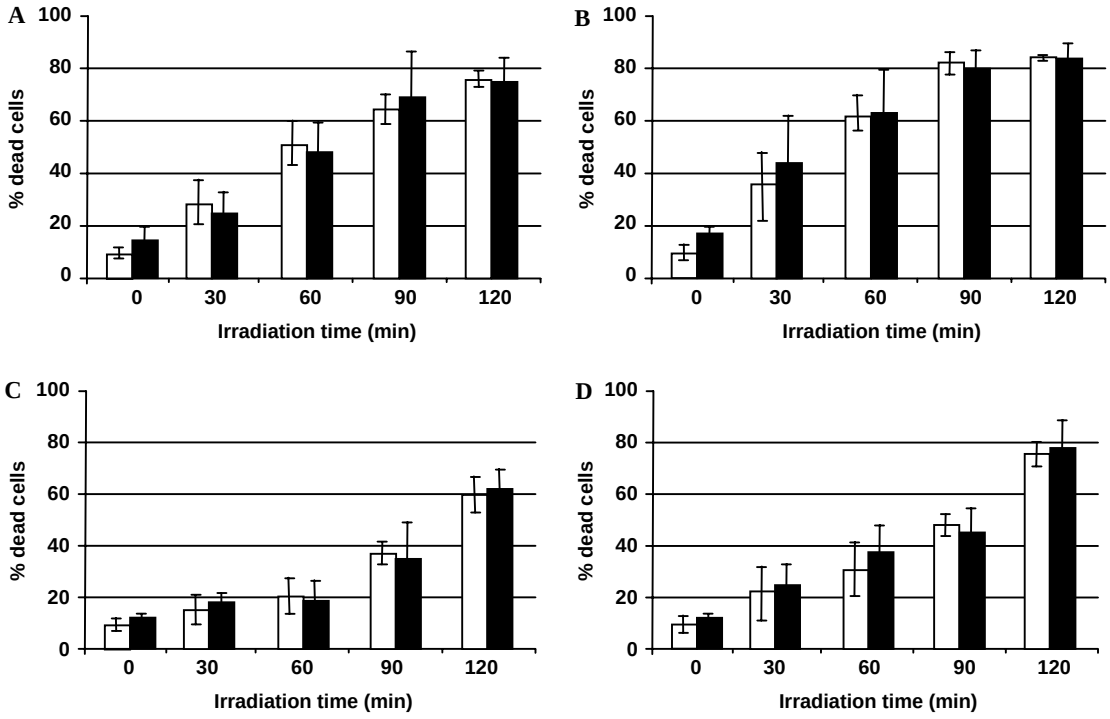


Figure 4. Cell irradiation and analysis were conducted as described in Experimental. Cells were incubated with tritolylporphyrin (2×10^{-6} M) with monopolyamine substituent in *ortho* position **16b** (A), **17b** (B) and in *para* position **16a**, **17a** (C and D). Dead cell count was estimated by flow cytometry just after irradiation (void bars) or after a further 24 h incubation in the dark (solid bars). Error bars are based upon standard deviations.

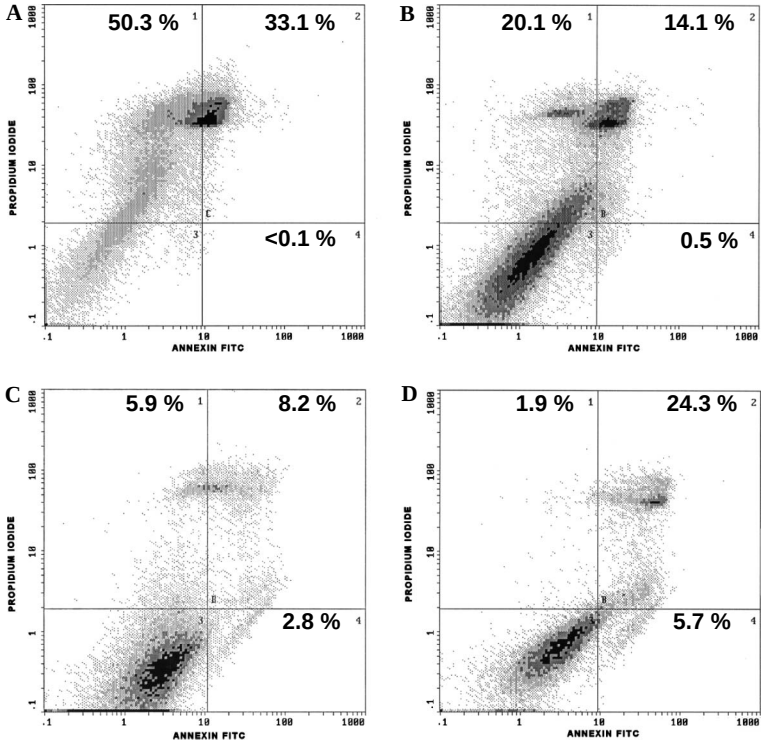


Figure 5. AnnexinV-FITC/propidium iodide assays were performed on cells irradiated for 30 min in the presence of porphyrin polyamine conjugate **19** at 2×10^{-6} M (A), 2×10^{-7} M (B) or 1.25 μg/mL Photofrin II® (D); (C) control cells without photosensitizer.

In order to evaluate the influence of polyamine units on the cell viability, biological assay with porphyrin conjugates devoid of free amino functions (compounds **12**, **13** with Boc-protective groups) or without spermine, spermidine (Protoporphyrin IX dimethylester) was realized (data not shown). These compounds induced 20% and

60% of cell death, respectively, after 120 min; this photoactivity is significantly reduced if compared with **18**, **19** So, the presence of polyamine with free amino functions on the macrocycle was essential to induce massive cell death. For tritolylporphyrins **16**–**17**, the position on the phenyl ring of one polyamine unit was different (*para* to *ortho*). Within the later series, photoactivity displayed by the *ortho* compounds was always significantly stronger than their *para* counterparts. It should be noted that these results are reminiscent of those previously observed with mono *ortho* and *para* β-glycosyl porphyrins.[19] On the other hand, the nature of polyamine units does not seem to be important; indeed, porphyrin conjugates bearing spermine or spermidine units showed the same activity.

Then and with the aim of correlating cell death mechanism with the structure of photosensitizers,[20] we have used Annexin V-FITC/PI staining and flow cytometry to characterize the cell death pathway, that is, the presence of apoptotic or necrotic cells.[21] Our experiments realized with amphiphilic compounds **18**, **19** showed a massive death probably corresponding to a rapid necrosis.

4. Conclusion

A series of new polyamine porphyrin conjugates have been designed, synthesized, and characterized. Preliminary in vitro tests confirm previous observations suggesting the requirement of amphiphilicity for efficient photodynamic activity. The binding of two polyamine units is obviously a good means to bring a balance between hydrophilicity and hydrophobicity. Massive cell death induced by these compounds after 30 min irradiation is likely the consequence of rapid necrotic death. Further in vitro experiments are presently conducted for evaluating other parameters as cellular uptake, subcellular binding of sensitizers (lysosomes, mitochondria, and/or cell membranes), and cell death pathway.

5. Experimental

5.1. General

All solvents and reagents were purchased from Aldrich, Prolabo or Acros. Pyrrole was distilled over CaH_2 under reduced pressure immediately before use. Dimethylformamide was distilled over CaH_2 under reduced pressure and stored under argon. Methylene chloride was distilled over P_2O_5 and then CaH_2. Protoporphyrin IX was purchased from Aldrich. Analytical thin-layer chromatography (TLC) was performed on silica gel Merck $60F_{254}$. Column chromatography was carried out with silica gel (60 ACC; 15–40 μm, Merck). ^1H and ^{13}C NMR spectroscopy was performed with a Brucker DPX-400 spectrometer. Chemical shifts are reported as δ ppm, downfield from internal TMS, and are listed according to the standard numbering of *meso*-arylporphyrins and protoporphyrin IX. UV–vis spectra were recorded on a Perkin-Elmer Lambda 25 double-beam spectrophotometer using 10 or 50 mm quartz cells. Infra-Red spectra were recorded on a Perkin-Elmer spectrum 1000 with KBr pellets. Mass spectrometry (ESI) was performed by the Centre Régional de Mesures Physiques de l'Ouest, CRMPO, Rennes. MALDI-TOF mass spectra were recorded with a Voyager Elite (Framingham MA, USA) time-of-flight mass spectrometer equipped with a 337 nm nitrogen laser (VSL 337ND). It was operated in the reflectron delayed extraction mode at an acceleration voltage of 20 kV. Internal standards (peptides) were used to calibrate the mass scale with the two- point calibration Software version 3.07.1 from PerSeptive Biosystems. One microliter of an acetone solution of matrix (α-cyano-4-hydroxycinnamic acid) and compounds at concentrations of 0.1 M and 0.01 mM, respectively, was deposited onto the stainless steel sample slide and, dried in air. Elemental analyses were carried by the 'Service Régional de Microanalyse de l'Université Pierre et Marie Curie, Paris.'

5.2. Synthesis

5.2.1. N^1,N^8-Bis-*tert*-butoxycarbonylspermidine 3. Spermidine (2.16 mL, 13.8 mmol, 1 equiv) was dissolved in 30 mL of anhydrous THF. A solution of 2-(*tert*-butyloxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON, 6.80 g, 27.6 mmol, 2 equiv) dissolved in 30 mL of anhydrous THF was added dropwise with constant stirring and then 5.77 mL of triethylamine (41.4 mmol, 3 equiv) was added. The reaction was carried out under an argon atmosphere and stirred during 16 h at 0 °C. The disappearance of spermidine **1** was monitored by TLC ($R_f = 0.1$). After evaporation under reduced pressure, residue was redissolved in methylene chloride (50 mL) and washed with 5% aqueous solution of sodium hydroxyde (2×50 mL) and water (3×50 mL). The organic layer was separated and dried over anhydrous magnesium sulfate, filtered, concentrated, and subjected to flash chromatography ($CHCl_3$/MeOH, 100:0 to 50:50) to give compound **3**, which was recrystallized in petroleum ether (3.76 g, 76%). $R_f = 0.56$ (ethyl acetate/acetone/acetic acid/water, 5:3:1:1). $T_F = 80$ °C (lit.[14] $T_F = 79$–80 °C). IR (KBr) 3373, 2977, 2929, 1685, 1165 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz) δ 1,44 (s, 18H), 1.57 (m, 2H), 1.68 (m, 2H), 1.85 (m, 2H), 2.80 (t, $J_{H,H} = 6.56$ Hz, 2H), 2.84 (t, $J_{H,H} = 6.56$ Hz, 2H), 3.13 (m, 2H), 3.25 (m, 2H), 4.90 (br s, 1H), 5.26 (br s, 1H). RMN ^{13}C ($CDCl_3$) δ, 156.1, 156.0, 78.9, 49.4, 47.7, 40.4, 39.2, 29.9, 28.4, 27.8, 27.4.

5.2.2. N^1,N^8,N^{12}-Tris-*tert*-butoxycarbonylspermine 4. Spermine (500 mg, 2.47 mmol, 1 equiv) was dissolved in 20 mL of anhydrous THF. A solution of 2-(*tert*-butyloxycarbonyloxyimino)-2-phenylacetonitrile (BOC-ON, 1.83 g, 7.41 mmol, 3 equiv) dissolved in 25 mL of anhydrous THF was added dropwise with constant stirring and then 1.38 mL of triethylamine (9.88 mmol, 4 equiv) was added. The reaction was carried out under an argon atmosphere and stirred for 20 h at 0 °C. The disappearance of spermine **2** was monitored by TLC ($R_f = 0.1$). After evaporation under reduced pressure, oil was redissolved in methylene chloride (50 mL) and washed with 5% aqueous solution of sodium hydroxyde (2×50 mL)

and water (3 × 50 mL). The organic layer was separated and dried over anhydrous magnesium sulfate, filtered, concentrated, and subjected to flash chromatography ($CHCl_3$/MeOH, 100:0 to 50:50) to give compound **4** (897 mg, 72%). R_f = 0.48 (ethyl acetate/acetone/acetic acid/water, 5:3:1:1). IR (KBr) 3347, 2976, 2932, 1694, 1172 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$) δ 1.44 (s, 27 H), 1.46 (m, 2H), 1.55 (m, 2H), 1.65 (m, 2H), 2.60 (t, $J_{H,H}$ = 7.0 Hz, 2H), 2.66 (t, $J_{H,H}$ = 6.8 Hz, 2H), 3.17 (m, 8H), 4.79 (br s, 1H), 5.15 (br s, 1H). RMN ^{13}C ($CDCl_3$) δ 156.1, 156.8, 155.4, 79.7, 79.5, 78.9, 49.6, 47.7, 46.8, 43.9, 39.2, 37.5, 30.0, 29.2, 28.5, 27.4, 26.5.

5.2.3. N^4-(4-Phthalimidobutyl)-N^1,N^8-bis-*tert*-butoxycarbonylspermidine 5. Compound **3** (875 mg, 2.48 mmol, 1 equiv) and *N*-(4-bromobutyl)phthalimide (875 mg, 3.10 mmol, 1.25 equiv) were dissolved in dry acetonitrile (20 mL) with an excess of K_2CO_3 (5 equiv). The mixture was stirred for 18 h under reflux. After completion of the reaction, acetonitrile was evaporated under vacuum and the crude product was dissolved in dichloromethane. The organic layer was washed with $NaHCO_3$ (2 × 50 mL) and water (2 × 50 mL), dried ($MgSO_4$), and then evaporated to afford, after purification by column chromatography (chloroform/ethanol, 100:0 to 80:20), 1.3 g of compound **5** (96%). R_f = 0.44 (CH_2Cl_2/EtOH, 90:10).IR (KBr) 3356, 2974, 2935, 1771, 1711, 1171 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$) δ 1.44 (s, 18H), 1.48 (m, 6H), 1.65 (m, 4H), 2.44 (m,6H), 3.14 (m, 4H), 3.70 (t, $J_{H,H}$ = 7.2 Hz, 2H), 4.91 (br s, 1H), 5.30 (br s, 1H), 7.71 (dd, $J_{H,H}$ = 3.1 Hz and $J_{H,H}$ = 5.4 Hz, 2H), 7.85 (dd, $J_{H,H}$ = 3.1 Hz and $J_{H,H}$ = 5.4 Hz, 2H). RMN ^{13}C ($CDCl_3$) δ 168.4, 156.1, 133.4, 132.1, 123.7, 79.5, 78.7, 53.6, 53.3, 52.3, 40.5, 39.9, 37.8, 29.7, 28.5, 26.9, 26.5, 24.5, 24.0. HRMS (ESI): calcd for $C_{29}H_{47}N_4O_6$: *m*/*z* 546.3416. Found: *m*/*z* 547.3496 $[M+H]^+$.

5.2.4. N^4-(4-Phthalimidobutyl)-N^1,N^8,N^{12}-tris-*tert*-butoxycarbonylspermine 6. Compound **4** (1.277 mg, 2.54 mmol, 1 equiv) and *N*-(4-bromobutyl)phthalimide (896 mg, 3.17 mmol, 1.25 equiv) were dissolved in dry acetonitrile (20 mL) with an excess of K_2CO_3 (5 equiv). The mixture was stirred for 18 h under reflux. The disappearance of **4** was monitored by TLC (CH_2Cl_2/EtOH, 90:10). Acetonitrile was evaporated under vacuum and the crude product was dissolved in dichloromethane. The organic layer was washed with $NaHCO_3$ (2 × 50 mL) and water (2 × 50 mL), dried ($MgSO_4$), and then evaporated to afford, after purification by column chromatography (chloroform/ethanol, 100:0 to 90:10), 1.7 g of compound **6** (95%). R_f = 0.49 (CH_2Cl_2/EtOH, 90:10). IR (KBr) 3354, 2975, 2932, 1772, 1713, 1170 cm^{-1}; ^1H NMR ($CDCl_3$, 400.13 MHz) δ 1.44 (m, 27H), 1.46 (m, 8H), 1.65 (m, 4H), 2.42 (m, 6H), 3.14 (m, 8H), 3.70 (m, 2H), 4.91 (br s, 1H), 5.34 (br s, 1H), 7.71 (dd, $J_{H,H}$ = 3.14 Hz and $J_{H,H}$ = 5.08 Hz, 2H), 7.84 (dd, $J_{H,H}$ = 3.13 Hz and $J_{H,H}$ = 5.08 Hz, 2H). RMN ^{13}C ($CDCl_3$) δ, 168.4, 156.0, 133.9, 132.1, 123.2, 79.5, 78.7, 77.2, 53.7, 53.4, 52.6, 46.9, 44.1, 43.9, 39.9, 37.8, 28.5, 28.4, 28.3, 27.0, 26.5, 26.0, 24.3. HRMS (ESI): calcd for $C_{37}H_{62}N_5O_8$: *m*/*z* 703.4518. Found: *m*/*z* 704.4592 $[M+H]^+$.

5.2.5. N^4-(4-Aminobutyl)-N^1,N^8-bis-*tert*-butoxycarbonylspermidine 7. Compound **5** (1.070 g, 1.96 mmol, 1 equiv) and an excess of hydrazine monohydrate (4.76 g, 98 mmol, 50 equiv) were dissolved in THF/MeOH (80/20) (12 mL). The mixture was stirred 5 h at 90 °C and then 18 h at 50 °C. After evaporation under vacuum, the residue was redissolved in methylene chloride (50 mL) and washed with 5% sodium hydroxide solution (2 × 50 mL) and water (3 × 50 mL). The organic phase was separated, dried over anhydrous magnesium sulfate, filtered, concentrated, and subjected to flash chromatography using 3% NH_4OH/CH_3OH to give the desired amine **7** (776 mg: 95%). R_f = 0.35 (MeOH + 5% NH_4OH). IR (KBr) 3347, 2974, 2933, 1693, 1173 cm^{-1};^1H NMR (400.13 MHz, $CDCl_3$) δ 1.44 (m, 26H), 1.60 (m, 2H), 2.40 (m, 6H), 2.70 (t, J = 6.4 Hz, 2H), 3.14 (m, 4H), 4.87 (br s, 1H), 5.53 (br s, 1H). ^{13}C RMN ($CDCl_3$) δ , 156.1, 79.5, 78.8, 53.9, 53.7, 52.1, 42.2, 40.5, 40.1, 29.6, 28.5 , 28.0, 26.7, 24.4, 24.3. HRMS (ESI): calcd for $C_{21}H_{44}N_4O_4$: *m*/*z* 416.3433. Found: *m*/*z* 417.3440 $[M+H]^+$.

5.2.6. N^4-(4-Aminobutyl)-N^1,N^8,N^{12}-tris-*tert*-butoxycarbonylspermine 8. Compound **6** (1.225 g, 1.78 mmol, 1 equiv) and an excess of hydrazine monohydrate (4.33 g, 89 mmol, 50 equiv) were dissolved in THF/MeOH (80/20) (15 mL). The mixture was stirred for 5 h at 90 °C and then for 18 h at 50 °C. After evaporation under vacuum, the residue was redissolved in methylene chloride (50 mL) and washed with 5% sodium hydroxide solution (2 × 50 mL) and water (3 × 50 mL). The organic phase was separated, dried over anhydrous magnesium sulfate, filtered, concentrated, and subjected to flash chromatography using 3% NH_4OH/CH_3OH to give the desired amine **8** (780 mg: 86%). R_f = 0.40 (MeOH + 5% NH_4OH). IR (KBr) 3353, 2974, 2932, 1693, 1172 cm^{-1}; ^1H NMR (400.13 MHz, $CDCl_3$) δ 1.43 (m, 35H), 1.60 (m, 4H), 2.40 (m, 6H), 2.70 (t, J = 6.4 Hz, 2H), 3.17 (m, 8H), 4.88 (br s, 1H), 5.36 (br s, 1H). ^{13}C RMN ($CDCl_3$) δ 156.1, 79.5, 78.8, 77. 3, 54.0, 53.7, 52.7, 46.9, 43.7, 42.1, 40.0 , 37.5, 31.6, 29.7, 28.5, 26.9, 24.5, 24.3. HRMS (ESI): calcd for $C_{29}H_{59}N_5O_6$: *m*/*z* 573.4536. Found: *m*/*z* 574.4544 $[M+H]^+$.

Monohydroxyphenylporphyrins **9a**,**b** were synthesized according to the literature.[11d]

5.2.7. General procedure for the synthesis of monocarboxypropyloxyphenylporphyrins. Porphyrins **9a**,**b** (1 equiv) were dissolved in dry DMF (10 mL) with a large excess of K_2CO_3 (20 equiv). The mixture was stirred for 15 min at room temperature. Ethyl 4-bromobutyrate (5 equiv) was added and then the solution was stirred at room temperature overnight in the dark. After reaction, DMF was evaporated under vacuum and the crude product was dissolved in methylene chloride. The organic layer was washed several times with water, dried ($MgSO_4$), and then evaporated to afford, after purification by thin-layer chromatography, the pure porphyrins **10a**,**b**.

Tritolyl derivatives **10a** or **b** were dissolved in DMF (8 mL) and KOH (2 mL, 1 M in ethanol) was added.

The mixture was stirred under reflux for 2 h. After cooling, solvent was evaporated under vacuum and the residue was dissolved in methylene chloride. The solution was neutralized by addition of HCl (1 M) washed with water and dried over magnesium sulfate. Column chromatography performed with $CHCl_3$ and increasing amounts of ethanol (0–10%) allowed purification of porphyrins **11a**,**b**.

5.2.8. 5-(4-[3-Ethoxycarbonylpropyloxy]phenyl)-10,15,20-tris(4-methylphenyl) porphyrin (10a). Porphyrin **9a** (150 mg, 0.22 mmol, 1 equiv), ethyl 4-bromobutyrate (159 mL, 1.1 mmol, 5 equiv), and K_2CO_3 (615 mg, 4.4 mmol, 20 equiv) afforded pure product **10a**, 167 mg, (90%). R_f = 0.56 ($CHCl_3$); UV–vis (CH_2Cl_2): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 420 (354.0), 516 (14.3), 552 (7.2), 592 (4.7), 648 (4.2); ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ −2,71 (br s, 2H, NH pyr),1.36 (t, 3H,$J_{H,H}$ = 7.2 Hz, CH_3 ethyl), 2.31 (m, 2H, H_β –CH_2–), 2.66 (m, 2H, H_β –CH_2–C═O), 2.69 (s, 9H, CH_3 tolyl), 4.25 (q, 2H, $J_{H,H}$ = 7.1 Hz, CH_2 ethyl), 4.30 (t, 2H, $J_{H,H}$ = 6.0 Hz, H_α –O–CH_2–), 7.26 (d, 2H, $J_{H,H}$ = 8.6 Hz, $H_{3,5}$ Ar), 7.56 (d, 6H, $J_{H,H}$ = 7.8 Hz, $H_{3,5}$ tolyl), 8.11 (d, 2H, $J_{H,H}$ = 7.7 Hz $H_{2,6}$ Ar), 8.12 (d, 6H, $J_{H,H}$ = 7.7 Hz, $H_{2,6}$ tolyl), 8.88 (s, 8H, H_β pyr.). MS (MALDI) *m*/*z* 787.9 ([M+H]$^+$ monoisotopic calcd: 786.4).

5.2.9. 5-(2-[3-Ethoxycarbonylpropyloxy]phenyl)-10,15,20-tris(4-methylphenyl) porphyrin (10b). Porphyrin **9b** (150 mg, 0.22 mmol, 1 equiv), ethyl 4-bromobutyrate (159 mL, 1.1 mmol, 5 equiv), and K_2CO_3 (615 mg, 4.4 mmol, 20 equiv) afforded pure product **10b**, 158 mg, (85%). R_f = 0.66 ($CHCl_3$); UV–vis (CH_2Cl_2): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3) 420 (391.0), 516 (13.4), 552 (8.1), 592 (4.6), 648 (2.8); ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ −2.72 (s, 2H, NH pyr), 0.73 (t, 3H,$J_{H,H}$ = 7.2 Hz, CH_3 ethyl), 1.30 (m, 4H, –CH_2-CH_2-C═O), 2.69 (s, 9H, CH_3 tolyl), 3.59 (q, 2H, $J_{H,H}$ = 7.2 Hz, CH_2 ethyl), 3.91 (br t, 2H, $J_{H,H}$ = 5.2 Hz, H –CH_2–), 7.30 (br d, 1H, $J_{H,H}$ = 8.0 Hz, H_4 Ar), 7.34 (br t, 1H, $J_{H,H}$ = 7.2 Hz, H_6 Ar), 7.53 (d, 6H, $J_{H,H}$ = 7.6 Hz, $H_{3,5}$ tolyl), 7.73 (dt, 1H, $J_{H,H}$ = 8.4–1.6 Hz H_5 Ar), 8.02 (dd, 1H, $J_{H,H}$ = 7.6–1.6 Hz, H_3 Ar), 8.10 (br d, 6H, $J_{H,H}$ = 8.0 Hz, $H_{2,6}$ tolyl), 8.77 (d, 2H, $J_{H,H}$ = 4.8 Hz, H_β pyr), 8.83 (d, 2H, $J_{H,H}$ = 4.7 Hz, H_β pyr), 8.84 (s, 4H, H_β pyr). MS (MALDI) *m*/*z* 787.9 ([M+H]$^+$ monoisotopic calcd: 786.4).

5.2.10. 5-(4-[3-Carboxypropyloxy]phenyl)-10,15,20-tris(4-methylphenyl) porphyrin (11a). The saponification of **10a** carried out on 100 mg gave porphyrin **11a** in 98% yield. R_f = 0.49 ($CHCl_3$/EtOH, 95:5); UV–vis (CH_2Cl_2): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3) 420 (355.7), 517 (13.4), 553 (7.2), 592 (4.0), 648 (3.8); ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ −2,76 (br s, 2H, NH pyr), 2.35 (m, 2H, H_β –CH_2–), 2.68 (s, 9H, CH_3 tolyl), 2.77 (t, 2H, $J_{H,H}$ = 7.1 Hz, H_γ –CH_2-C═O), 4.31 (t, 2H, $J_{H,H}$ = 6.1 Hz, H_α O-CH_2-), 4.30 (t, 2H, $J_{H,H}$ = 6.0 Hz, H_α –O–CH_2–), 7.23 (m, 2H, $H_{3,5}$ Ar), 7.52 (br d, 6H, $J_{H,H}$ = 7.8 Hz, $H_{3,5}$ tolyl), 8.07 (d, 2H, $J_{H,H}$ = 7.7 Hz $H_{2,6}$ Ar), 8.09 (d, 6H, $J_{H,H}$ = 7.6 Hz, $H_{2,6}$ tolyl), 8.83 (br s, 8H, H_β pyr). MS (MALDI) *m*/*z* 759.9 ([M+H]$^+$ monoisotopic calcd: 758.3).

5.2.11. 5-(2-[3-Carboxypropyloxy]phenyl)-10,15,20-tris(4-methylphenyl) porphyrin (11b). The saponification of **10b** carried out on 120 mg gave porphyrin **11b** in 97% yield. R_f = 0.49 ($CHCl_3$/EtOH, 95:5); UV–vis (CH_2Cl_2): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3) 420 (388.6), 517 (15.7), 553 (9.8), 592 (4.9), 648 (4.2); ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ −2,76 (br s, 2H, NH pyr), 2.68 (s, 9H, CH_3 tolyl), 7.23 (m, 2H, $H_{3,5}$ Ar), 7.52 (br d, 6H, $J_{H,H}$ = 7.8 Hz, $H_{3,5}$ tolyl), 8.07 (d, 2H,$J_{H,H}$ = 7.7 Hz $H_{2,6}$ Ar), 8.09 (d, 6H, $J_{H,H}$ = 7.6 Hz, $H_{2,6}$ tolyl), 8.83 (br s, 8H, H_β pyr). MS (MALDI) *m*/*z* 759.8 ([M+H]$^+$ monoisotopic calcd: 758.3).

5.2.12. General procedure for the synthesis of porphyrins bearing spermine or spermidine units. N^4-(4-Aminobutyl)-N^1,N^8-bis-*tert*-butoxycarbonylspermidine **7** (1.1 equiv), or N^4-(4-aminobutyl)-N^1, N^8,N^{12}-tris-*tert*-butoxycarbonylspermine **8** (1.1 equiv) was dissolved in DMF. A solution of carboxy-porphyrins **11a**,**b** (1 equiv) or protoporphyrin IX (0.5 equiv) and *N*,*N*′-dicyclohexylcarbodiimide (DCC) (1.1 equiv) in dry DMF (1 mL) was added. After addition of 1-hydroxybenzotriazole (HOBt) (1.1 equiv), the mixture was kept at room temperature, in the dark, under argon, for 42 or 72 h (protoporphyrin IX). DMF was evaporated under vacuum and the crude product was dissolved in dichloromethane. The organic layer was washed with water (2 × 50 mL), dried $MgSO_4$, and then evaporated to afford, after purification by thin-layer chromatography, the pure product.

5.2.13. 5-(4-[N^4-(4-Amidobutyl)-N^1, N^8-bis-*tert*-butoxycarbonylspermidine-3-amidopropyloxy]phenyl)-10,15,20-tris(4-methylphenyl) porphyrin (12a). Porphyrin **11a** (52 mg, 68.6 μmol, 1 equiv) and compound **7** (31 mg, 72.5 μmol, 1.1 equiv) reacted with DCC (16 mg, 68.6 μmol, 1.1 equiv) and 1-hydroxybenzotriazole (HOBt) (10 mg, 68.6 μmol, 1.1 equiv) to afford pure product **12a**, 65 mg (81%). R_f = 0.63 (CH_2Cl_2/EtOH, 70:30). UV–vis (CH_2Cl_2): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3) 420 (420.3), 516 (14.3), 553 (12.2), 592 (9.4), 648 (8.2). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ −2,76 (br s, 2H, NH pyr), 1.43 (m, 18H, C**H**$_3$ Boc), 1.49 (m, 4H, N-CH_2-(C**H**$_2$)$_2$-CH_2-NHBoc), 1.57 (quint, 2H, $J_{H,H}$ = 6.0 Hz, N-CH_2-C**H**$_2$-CH_2-NHBoc), 1.65 (m, 4H, CO-NH-CH_2-(C**H**$_2$)$_2$-CH_2-N), 2.29 (quint, 2H, Porph-O-CH_2-C**H**$_2$–), 2.53 (t, 2H, $J_{H,H}$ = 7.4 Hz, CO-NH-C**H**$_2$-$(CH_2)_3$-N),2.65-2.69 (m, 6H, Porph-O-$(CH_2)_2$-C**H**$_2$-C═O, N-C**H**$_2$-$(CH_2)_2$-NHBoc and N-C**H**$_2$-$(CH_2)_3$-NHBoc), 2.70 (s, 9H, CH_3 tolyl), 3.09 (m, 2H, N-$(CH_2)_3$-C**H**$_2$-NHBoc), 3.16 (m, 2H, N-$(CH_2)_2$-C**H**$_2$-NHBoc), 3.31 (m, 2H, CO-NH-$(CH_2)_3$-C**H**$_2$-N), 4.27 (t, 2H, $J_{H,H}$ = 6.0 Hz, Porph-O-C**H**$_2$–), 4.74 (br s, 1H, N**H**Boc), 5.30 (br s, 1H, N**H**Boc), 7.25 (d, 2H, $J_{H,H}$ = 8.4 Hz, $H_{3,5}$ Ar), 7.53 (d, 6H, $J_{H,H}$ = 7.8 Hz, $H_{3,5}$ tolyl), 8.08 (d, 6H, $J_{H,H}$ = 7.8 Hz, $H_{2,6}$ tolyl), 8.10 (d, 2H,$J_{H,H}$ = 8.4 Hz $H_{2,6}$ Ar), 8.85 (s, 8H, H_β pyr). MS (MALDI) *m*/*z* 1157.5 ([M+H]$^+$ monoisotopic calcd: 1156.7).

5.2.14. 5-(2-[N^4-(4-Amidobutyl)-N^1, N^8-bis-*tert*-butoxycarbonylspermidine-3-amidopropyloxy]phenyl)-10,15,20-tris(4-methylphenyl) porphyrin (12b). Porphyrin **11b** (41 mg, 54.1 μmol, 1 equiv) and compound **7** (25 mg,

59.5 μmol, 1.1 equiv) reacted with DCC (12 mg, 59.5 μmol, 1.1 equiv) and 1-hydroxybenzotriazole (HOBt) (8 mg, 59.5 μmol, 1.1 equiv) to afford pure product **12b**, 57 mg (77%). R_f = 0.41 (CH_2Cl_2/EtOH, 85:15). UV–vis (CH_2Cl_2): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3) 419 (487.9), 516 (16.0), 553 (10.5), 593 (6.1), 648 (4.3). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ −2,75 (br s, 2H, NH pyr), 1.13–1.25 (m, 12H, Porph-O-CH_2-(C**H**$_2$)$_2$-CONH-CH_2-(C**H**$_2$)$_2$-CH_2-N and N-CH_2-(C**H**$_2$)$_2$-CH_2-NHBoc). 1.34 (m, 18H, C**H**$_3$ Boc), 1.42 (m, 2H, N-CH_2-C**H**$_2$-CH_2-NHBoc), 1.91–2.00 (m, 6H, CONH-C**H**$_2$-$(CH_2)_3$-N, N-C**H**$_2$-$(CH_2)_3$-NHBoc and N-C**H**$_2$-$(CH_2)_2$-NHBoc), 2.64–2.87(m, 6H, N-CH_2-CH_2-C**H**$_2$-NHBoc, N-$(CH_2)_3$-C**H**$_2$-NHBoc and CONH-$(CH_2)_3$-C**H**$_2$-N), 2.70 (s, 9H, CH_3 tolyl), 3.82 (t, 2H, $J_{H,H}$ = 5.5 Hz, Porph-O-C**H**$_2$-$(CH_2)_2$-CONH–), 4.73 (br s, 1H, N**H**Boc), 5.29 (br s, 1H, N**H**Boc), 7.29 (br d, 1H, $J_{H,H}$ = 8.2 Hz, H_3 Ar), 7.40 (br t, 1H, $J_{H,H}$ = 7.3 Hz, H_5 Ar) 7.55 (m, 6H, $H_{3,5}$ tolyl), 7.75 (dt, 1H, $J_{H,H}$ = 8.3–1.5 Hz, H_4 Ar), 8.06 (m, 6H, $H_{2,6}$ tolyl), 8.13 (m, 1H, H_6 Ar), 8.87 (m, 8H, H_β pyr). MS (MALDI) *m*/*z* 1157.6 ([M+H]$^+$ monoisotopic calcd: 1156.7).

5.2.15. 5-(4-[N^4-(4-Aminobutyl)-N^1,N^8,N^{12}-bis-*tert*-butoxycarbonylspermine-3-amidopropyloxy]phenyl)-10,15,20-tris(4-methylphenyl) porphyrin (13a). Porphyrin **11a** (106 mg, 0.14 mmol, 1 equiv) and compound **8** (86 mg, 0.15 mmol, 1.1 equiv) reacted with DCC (31 mg, 0.15 mmol, 1.1 equiv) and 1-hydroxybenzotriazole (HOBt) (20 mg, 0.15 mmol, 1.1 equiv) to afford pure product **13a**, 65 mg (74%). R_f = 0.48 (CH_2Cl_2/EtOH, 80:20). UV–vis (CH_2Cl_2): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 422 (460.6), 517 (18.7), 553 (14.6), 595 (6.5), 648 (4.4). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ −2,76 (br s, 2H, NH pyr), 1.44 (m, 18H, C**H**$_3$ Boc), 1.51 (m, 4H, N-CH_2-(C**H**$_2$)$_2$-CH_2-NH-$(CH_2)_3$-NHBoc), 1.57 (m, 4H, N-CH_2-C**H**$_2$-CH_2-NH-CH_2-C**H**$_2$-CH_2-NHBoc), 1.65 (m, 4H, CO-NH-CH_2-(C**H**$_2$)$_2$-CH_2-N), 2.30 (quint, 2H, $J_{H,H}$ = 6.2 Hz, Porph-O-CH_2-C**H**$_2$–), 2.53(m, 6H, CO-NH-C**H**$_2$-$(CH_2)_3$-N, N-C**H**$_2$-$(CH_2)_3$NH-C**H**$_2$-$(CH_2)_2$-NHBoc), 2.67 (m, 2H, Porph-O-$(CH_2)_2$-C**H**$_2$-C=O), 2.69 (s, 9H, CH_3 tolyl), 3.08 (m, 2H, N-$(CH_2)_3$-C**H**$_2$-NH-$(CH_2)_3$-NHBoc), 3.17 (m, 6H, N-$(CH_2)_2$-C**H**$_2$-NHBoc and -$(CH_2)_4$-NH-C**H**$_2$-CH_2-C**H**$_2$-NHBoc), 3.31 (m, 2H, CO-NH-$(CH_2)_3$-C**H**$_2$-N), 4.28 (t, 2H, $J_{H,H}$ = 5.9 Hz, Porph-O-C**H**$_2$–), 4.74 (br s, 1H, N**H**Boc), 5.30 (br s, 1H, N**H**Boc), 7.25 (d, 2H, $J_{H,H}$ = 8.2 Hz, $H_{3,5}$ Ar), 7.54 (d, 6H, $J_{H,H}$ = 7.7 Hz, $H_{3,5}$ tolyl), 8.08 (d, 6H, $J_{H,H}$ = 7.7 Hz, $H_{2,6}$ tolyl), 8.10 (d, 2H,$J_{H,H}$ = 8.2 Hz $H_{2,6}$ Ar), 8.85 (s, 8H, H_β pyr). MS (MALDI) *m*/*z* 1314.6 ([M+H]$^+$ monoisotopic calcd: 1313.8).

5.2.16. 5-(4-[N^4-(4-Aminobutyl)-N^1,N^8,N^{12}-bis-*tert*-butoxycarbonylspermine-3-amidopropyloxy]phenyl)-10,15,20-tris(4-methylphenyl) porphyrin (13b). Porphyrin **11b** (35 mg, 46.15 μmol, 1 equiv) and compound **8** (29 mg, 50.77 μmol, 1.1 equiv) reacted with DCC (10 mg, 50.8 μmol, 1.1 equiv) and 1-hydroxybenzotriazole (HOBt) (7 mg, 50.8 μmol, 1.1 equiv) to afford pure product **13b**, 46 mg (76%). R_f = 0.48 (CH_2Cl_2/EtOH, 80:20). UV–vis (CH_2Cl_2): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3) 420 (446.4), 516 (14.4), 554 (9.0), 594 (5.6), 645 (3.7). ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C): δ −2.74 (br s, 2H, NH pyr), 1.08–1.25 (m, 12H, Porph-O-CH_2-(C**H**$_2$)$_2$-CONH-CH_2-(C**H**$_2$)$_2$-CH_2-N and N-CH_2-(C**H**$_2$)$_2$-CH_2-NH-$(CH_2)_4$-NHBoc). 1.32 (m, 18H, C**H**$_3$ Boc), 1.43 (m, 4H, N-CH_2-C**H**$_2$-CH_2-NHBoc and –$(CH_2)_4$NH-CH_2-C**H**$_2$-CH_2-NHBoc), 1.91–1.93 (m, 4H, CONH-C**H**$_2$-$(CH_2)_3$-N and N-C**H**$_2$-$(CH_2)_3$-NHBoc), 2.70 (s, 9H, CH_3 tolyl), 3.83 (t, 2H, $J_{H,H}$ = 5.4 Hz, Porph-O-C**H**$_2$-$(CH_2)_2$-CONH-), 2.62 (m, 2H, CONH-$(CH_2)_3$-C**H**$_2$-N), 2.86 (m, 2H,-$(CH_2)_4$NH-$(CH_2)_2$-C**H**$_2$-NHBoc) 3.11–3.16 (m, 6H, CO-NH-$(CH_2)_3$-C**H**$_2$-NH-C**H**$_2$-$(CH_2)_2$-NHBoc and N-$(CH_2)_2$-CH_2-NHBoc) 4.74 (br s, 1H, N**H**Boc), 5.31 (br s, 1H, N**H**Boc), 7.29 (br d, 1H, $J_{H,H}$ = 8.3 Hz, H_3 Ar), 7.39 (br t, 1H, $J_{H,H}$ = 7.3 Hz, H_5 Ar) 7.55 (d, 6H, $J_{H,H}$ = 7.5 Hz, $H_{3,5}$ tolyl), 7.76 (br t, 1H, $J_{H,H}$ = 8.2–1.6 Hz, H_4 Ar), 8.06 (m, 6H, $H_{2,6}$ tolyl), 8.13 (m, 1H, H_6 Ar), 8.86 (m, 8H, H_β pyr). MS (MALDI) *m*/*z* 1314.7 ([M+H]$^+$ monoisotopic calcd: 1313.8).

5.2.17. 13,17-di-[3-Amidoethyl-N^4-(4-aminobutyl)-N^1,N^8-bis-*tert*-butoxycarbonylspermidine]-2,7,12,18-tetramethyl-8,13-divinyl-porphyne (14). Protoporphyrin IX (70 mg, 0.12 mmol, 0.5 equiv) and compound **7** (108.3 mg, 0.26 mmol, 1.1 equiv) reacted with DCC (53.6 mg, 0.26 mmol, 1.1 equiv) and 1-hydroxybenzotriazole (HOBt) (35.1 mg, 0.26 mmol, 1.1 equiv) in DMF (20 mL). After 72 h of reaction and treatment, 132 mg (80%) of compound **14** was obtained after thin-layer chromatography (CH_2Cl_2/EtOH, 80:20 + 1% Et_3N). R_f = 0.45 (ethyl acetate/acetone/acetic acid/water, 5:3:1:1). UV–vis (CH_2Cl_2): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 407(113.9); 504(8.8); 541 (8.4); 575 (5.3); 629(3.2). ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ (ppm) = −3.69 (s, 2H, N**H** pyrrole), 1.36 (s, 36H, C**H**$_3$ Boc), 1.43–1.20 (m, 20H, –CONHCH$_2$(C**H**$_2$)$_2$ $CH_2N(CH_2)_2$, $(CH_2)_2NCH_2$C**H**$_2$C**H**$_2$$CH_2$NHBoc and –$(CH_2)_2NCH_2$ C**H**$_2$$CH_2$NHBoc), 3.1–2.2 (m, 24H, C**H**$_2$-N, C**H**$_2$-NH-), 3.50 (m, 16H, Proto-CH_2-C**H**$_2$-CO and C**H**$_3$β pyrrole), 4.26 (m, 4H, Proto-C**H**$_2$-CH_2-CO), 4.79 (s, 2H, –N**H**CO), 5.27 (s, 4H, N**H**Boc), 6.21 (bd, 2H, J = 10.7 Hz, C**H**$_2$ vinyl), 6.34 (d, 2H, J = 17.8 Hz, C**H**$_2$ vinyl), 8.14 (dd, 2H, J = 11.6–17.7 Hz, C**H** vinyl), 9.76, 9.69, 9.54 (s, 4H, **H**-*meso*). Anal. calcd for $C_{76}H_{118}N_{12}O_{10}$: C, 67.12; H, 8.75; N, 12.36. Found: C, 67.01; H, 8.63; N, 12.25. MS (MALDI) *m*/*z* 1360.4 ([M+H]$^+$ monoisotopic calcd: 1359.8).

5.2.18. 13,17-di-[3-Amidoethyl-N^4-(4-aminobutyl)-N^1,N^8,N^{12}-bis-*tert*-butoxycarbonylspermine]-2,7,12,18-tetramethyl-8,13-divinyl- porphyne (15). Protoporphyrin IX (55 mg, 0.97 mmol, 0.5 equiv) and compound **8** (108.3 mg, 0.21 mmol, 1.1 equiv) reacted with DCC (44 mg, 0.21 mmol, 1.1 equiv) and 1-hydroxybenzotriazole (HOBt) (29 mg, 0.21 mmol, 1.1 equiv) in DMF (20 mL). After 72 h of reaction and treatment, 139 mg (85%) of compound **15** was obtained after thin-layer chromatography (CH_2Cl_2/EtOH, 80:20 + 1% Et_3N). R_f = 0.62 (ethyl acetate/acetone/acetic acid/water, 5:3:1:1). UV–vis (CH_2Cl_2): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 406 (133.5); 504 (10.5); 540 (9.3); 574 (6.6); 629 (3.7). ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ (ppm) = −3.71(s, 2H, N**H**-pyrrole), 1.45–1.26

(m, 74H, C**H**$_3$ Boc, CONH-CH$_2$(C**H**$_2$)$_2$CH$_2$N(CH$_2$)$_2$, (CH$_2$)$_2$NCH$_2$C**H**$_2$C**H**$_2$CH$_2$NHBoc, –(CH$_2$)$_2$NCH$_2$C**H**$_2$ CH$_2$-NHBoc and (CH$_2$)$_2$NCH$_2$C**H**$_2$CH$_2$N(CH$_2$)), 3.22–2.88 (m, 32H, C**H**$_2$-N, –(C**H**$_2$)$_2$N-CO and C**H**$_2$NHCO), 3.71–3.46 (m, 16H, C**H**$_{3\beta}$ pyrrole and Proto-CH$_2$-C**H**$_2$-CO), 4.33 (m, 4H, Proto-C**H**$_2$-CH$_2$-CO), 4.75 (bs, 2H, -N**H**CO), 5.25 (bs, 4H, N**H**Boc), 6.21 (d, 2H, J = 11.4 Hz, C**H**$_2$ vinyl), 6.36 (d, 2H, J = 17.8 Hz, C**H**$_2$ vinyl), 8.19 (dd, 2H, J = 11.6–17.7 Hz, C**H** vinyl), 9.94, 9.90, 9.88, 9.75 (s, 4H, **H**-*meso*). Anal. calcd for $C_{92}H_{148}N_{14}O_{14}$: C, 66.00; H, 8.91; N, 11.71. Found: C, 65.83; H, 8.78; N, 11.59. MS (MALDI) *m*/*z* 1674.7 ([M+H]$^+$ monoisotopic calcd: 1374.2).

5.2.19. General procedure for the 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).

5.2.20. 5-(4-[*N*^{4}-(4-Amidobutyl)spermidine-3-amidopropyloxy]phenyl)-10,15,20-tris(4-methylphenyl) porphyrin (16a). R_f = 0.58 (CH_3CN/H_2O, 7:3 + 1% TFA). UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 416 (234.1); 515 (7.9); 547 (5.1); 590 (4.1); 650 (3.1). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$, 9:1, 25 °C): δ 1.58 (m, 4H, N-CH$_2$-(C**H**$_2$)$_2$-CH$_2$-NH$_2$), 1.78(m, 4H, CO-NH-CH$_2$-(C**H**$_2$)$_2$-CH$_2$-N), 1.99 (quint, 2H, J = 6.6 Hz, N-CH$_2$-C**H**$_2$-CH$_2$-NH$_2$) 2.29 (quint, 2H, J = 6.7 Hz, Porph-O-CH$_2$-C**H**$_2$-CH$_2$), 2,60(t, 2H, $J_{H,H}$ = 6.6 Hz, N-C**H**$_2$-(CH$_2$)$_3$-NH$_2$), 2.66 (m, 4H, Porph-O-(CH$_2$)$_2$-C**H**$_2$-CO-NH-C**H**$_2$-(CH$_2$)$_3$-N), 2.70 (s, 9H, CH$_3$ tolyl), 2.84 (t, 2H, $J_{H,H}$ = 6.6 Hz, N-C**H**$_2$-(CH$_2$)$_2$-NH$_2$), 2.97 (t, 2H, $J_{H,H}$ = 6.1 Hz, N-(CH$_2$)$_3$-C**H**$_2$-NH$_2$), 3.11 (t, 2H, $J_{H,H}$ = 6.4 Hz, N-(CH$_2$)$_2$-C**H**$_2$-NH$_2$) 3.30 (t, 2H, $J_{H,H}$ = 6.5 Hz, CO-NH-(CH$_2$)$_3$-C**H**$_2$-N), 4.30 (t, 2H, $J_{H,H}$ = 6.1 Hz, Porph-O-C**H**$_2$–), 7.27 (d, 2H, $J_{H,H}$ = 8.4 Hz, H$_{3,5}$ Ar), 7.55 (d, 6H, $J_{H,H}$ = 7.8 Hz, H$_{3,5}$ tolyl), 8.08 (d, 6H, H$_{2,6}$ tolyl, $J_{H,H}$ = 7.8 Hz), 8.10(d, 2H, H$_{2,6}$ Ar, $J_{H,H}$ = 8.4 Hz), 8.86 (br s, 8H, H$_\beta$ pyr). Anal. calcd for $C_{62}H_{68}N_8O_2$: C, 77.85; H, 7.17; N, 11.71. Found: C, 77.69; H, 7.08; N, 11.59. MS (MALDI) *m*/*z* 957.6 ([M+H$^+$] monoisotopic calcd: 956.5).

5.2.21. 5-(2-[*N*^{4}-(4-Amidobutyl)spermidine-3-amidopropyloxy]phenyl)-10,15,20-tris(4-methylphenyl) Porphyrin (16b). R_f = 0.45 (CH_3CN/H_2O 7/3 + 1% TFA). UV–vis (MeOH): λ_{max}, nm (ε, L.cm^{-1} mol^{-1} × 10^3): 415 (162.7); 515 (9.6); 550 (6.3); 591 (3.9); 648(3.4). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 9/1, 25 °C): δ 1.13 (m, 4H, N-CH$_2$-(C**H**$_2$)$_2$-CH$_2$-NH$_2$), 1.20-1.37 (m, 10H, Porph-O-CH$_2$-C**H**$_2$-C**H**$_2$-CO-NH-CH$_2$-(C**H**$_2$)$_2$-CH$_2$-N and N-CH$_2$-C**H**$_2$-CH$_2$-NH$_2$), 2.03-2.10 (m, 6H, N-C**H**$_2$-(CH$_2$)$_3$-NH$_2$ and CO-NH-C**H**$_2$-(CH$_2$)$_3$-N and N-C**H**$_2$-(CH$_2$)$_2$-NH$_2$), 2.66 (m, 2H, CO-NH-(CH$_2$)$_3$-C**H**$_2$-N), 2.71 (s, 9H, CH$_3$ tolyl), 2.75 (t, 2H, $J_{H,H}$ = 7.0 Hz, N-(CH$_2$)$_3$-C**H**$_2$-NH$_2$), 2.81 (t, 2H, $J_{H,H}$ = 7.0 Hz, N-(CH$_2$)$_2$-CH$_2$-NH$_2$), 3.96 (t, 2H, $J_{H,H}$ = 5.4 Hz, Porph-O-C**H**$_2$-), 7.43 (d, 1H, $J_{H,H}$ = 8.4 Hz, H$_3$ Ar), 7.39 (m, 1H, H$_5$ Ar), 7.59 (m, 6H, H$_{3,5}$ tolyl), 7.80 (dt, 1H, $J_{H,H}$ = 8.2–1.4 Hz, H$_4$ Ar), 8.04 (dd, 1H, $J_{H,H}$ = 7.6-1.4 Hz, H$_6$ Ar), 8.07 (m, 6H, H$_{2,6}$ tolyl), 8.87 (m, 8H, H$_\beta$ pyr). Anal. Calcd.for $C_{62}H_{68}N_8O_2$: C, 77.85; H, 7.17; N, 11.71. Found: C, 77.51; H, 7.01; N, 11.52. MS (MALDI) *m*/*z* 957.6 ([M+H$^+$] monoisotopic calcd: 956.5).

5.2.22. 5-(4-[*N*^{4}-(4-Aminobutyl)spermine-3-amidopropyloxy]phenyl)-10, 15,20-tris(4-methylphenyl) porphyrin (17a). R_f = 0.60 (CH_3CN/H_2O 7/3 + 1% TFA). UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 415(258.4); 514(12.7); 549(6.9); 590 (4.6); 646(3.7). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 9/1, 25 °C): δ 1.67(m, 6H, N-CH$_2$-(C**H**$_2$)$_2$-CH$_2$-NH-CH$_2$-CH$_2$-CH$_2$-NH$_2$), 1.84 (m, 6H, CO-NH-CH$_2$-(C**H**$_2$)$_2$-CH$_2$-N and N-CH$_2$-CH$_2$-C**H**$_2$-NH$_2$), 2.30 (quint, 2H, $J_{H,H}$ = 6.7 Hz, Porph-O-CH$_2$-C**H**$_2$-CH$_2$), 2.60 (t, 2H, $J_{H,H}$ = 7.1 Hz, CO-NH-C**H**$_2$-(CH$_2$)$_3$-N), 2.67 (t, 2H, $J_{H,H}$ = 6.6 Hz, Porph-O-(CH$_2$)$_2$-C**H**$_2$-C=O), 2.71 (s, 9H, C**H**$_3$ tolyl), 2.79 (m, 2H, N-C**H**$_2$-(CH$_2$)$_2$-NH-), 2.89 (t, 2H, $J_{H,H}$ = 6.4 Hz, N-C**H**$_2$-(CH$_2$)$_2$-NH$_2$), 3.10 (m, 6H, N-(CH$_2$)$_3$-C**H**$_2$-NH-(CH$_2$)$_2$-C**H**$_2$-NH$_2$ and N-(CH$_2$)$_2$-C**H**$_2$-NH$_2$), 3.24 (t, 2H, $J_{H,H}$ = 6.0 Hz, N-(CH$_2$)$_4$-NH-C**H**$_2$-(CH$_2$)$_2$-NH$_2$) 3.35 (m, 2H, CO-NH-(CH$_2$)$_3$-C**H**$_2$-N), 4.32 (t, 2H, $J_{H,H}$ = 6.0 Hz, Porph-O-C**H**$_2$-), 7.32 (d, 2H, $J_{H,H}$ = 8.3 Hz, H$_{3,5}$ Ar), 7.58 (d, 6H, $J_{H,H}$ = 7.7 Hz, H$_{3,5}$ tolyl), 8.07 (d, 6H, H$_{2,6}$ tolyl, $J_{H,H}$ = 7.7Hz), 8.12 (d, 2H, H$_{2,6}$ Ar, $J_{H,H}$ = 8.3 Hz), 8.87 (br s, 8H, H$_\beta$ pyr). Anal. calcd for $C_{65}H_{75}N_9O_2$: C, 77.01; H, 7.46; N, 12.44. Found: C, 76.84; H, 7.21; N, 12.09. MS (MALDI) *m*/*z* 1014.5 ([M+H$^+$] monoisotopic calcd: 1013.60).

5.2.23. 5-(2-[*N*^{4}-(4-Aminobutyl)spermine-3-amidopropyloxy]phenyl)-10, 15,20-tris(4-methylphenyl) porphyrin (17b). R_f = 0.64 (CH_3CN/H_2O 7/3 + 1% TFA). UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 415(143.1); 515(10.7); 550 (7.7); 591 (5.9); 648(5.3). ^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$ 9/1, 25 °C): δ 1.18-1.26 (m, 12H, Porph-O-CH$_2$-C**H**$_2$-C**H**$_2$-CO-NH-CH$_2$-(C**H**$_2$)$_2$-CH$_2$-N and N-CH$_2$-(C**H**$_2$)$_2$-CH$_2$-NH-), 1.37 (m, 4H, N-(CH$_2$)$_4$-NH-CH$_2$-C**H**$_2$-CH$_2$-NH$_2$ and N-CH$_2$-C**H**$_2$-CH$_2$-NH$_2$), 2.15 (m, 6H, N-C**H**$_2$-(CH$_2$)$_3$NH-, CO-NH-C**H**$_2$- and N-C**H**$_2$-(CH$_2$)$_2$-NH$_2$), 2.65 (m, 2H, CO-NH-(CH$_2$)$_3$-C**H**$_2$-N), 2.71 (s, 9H, CH$_3$ tolyl), 2.78 (m, 6H, N-(CH$_2$)$_3$-C**H**$_2$-NH-, N-(CH$_2$)$_2$-C**H**$_2$-NH$_2$ and –(CH$_2$)$_4$-NH-(CH$_2$)$_2$-C**H**$_2$-NH$_2$), 2.90 (m, 2H, -(CH$_2$)$_4$-NH-C**H**$_2$-(CH$_2$)$_2$-NH$_2$), 3.97 (t, 2H, $J_{H,H}$ = 5.4 Hz, Porph-O-C**H**$_2$–), 7.43 (d, 1H, $J_{H,H}$ = 8.4 Hz, H$_3$ Ar), 7.40 (m, 1H, H$_5$ Ar), 7.61 (m, 6H, H$_{3,5}$ tolyl), 7.81 (dt, 1H, $J_{H,H}$ = 8.4–1.4 Hz, H$_4$ Ar), 8.04 (dd, 1H, $J_{H,H}$ = 7.7–1.4 Hz, H$_6$ Ar), 8.09 (m, 6H, H$_{2,6}$ tolyl), 8.87 (br s, 8H, H$_\beta$ pyr) Anal. calcd for $C_{65}H_{75}N_9O_2$: C, 77.01; H, 7.46; N, 12.44. Found: C, 76.89; H, 7.38; N, 12.11. MS (MALDI) *m*/*z* 1014.5 ([M+H$^+$] monoisotopic calcd: 1013.6).

5.2.24. 13,17-di-[3-Amidoethyl-*N*^{4}-(4-aminobutyl)spermidine]-2,7,12,18-tetramethyl-8, 13-divinyl-porphine (18). R_f = 0.58 (CH_3CN/H_2O 7:3 + 1% TFA). UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 403 (27.0); 503 (2.5); 538 (2.1); 575 (1.3); 629 (0.9). ^{1}H NMR (CD_3OD, 400.13 MHz): δ (ppm) = 1.55–1.16 (m, 20H, –CONHCH$_2$(C**H**$_2$)$_2$CH$_2$N(CH$_2$)$_2$, (CH$_2$)$_2$NCH$_2$C**H**$_2$ C**H**$_2$CH$_2$NH$_2$ and –(CH$_2$)$_2$NCH$_2$C**H**$_2$CH$_2$NH$_2$), 3.01–2.64 (m, 16H, C**H**$_2$-N, C**H**$_2$-NH–), 3.34 (s, 4H, Proto-CH$_2$-C**H**$_2$-CO), 3.50 (m, 12H, C**H**$_{3\beta}$ pyrrole), 4.29 (m, 4H, Proto-C**H**$_2$-CH$_2$-CO), 6.40–6.30 (m, 4H, C**H**$_2$

vinyl), 8.09 (dd, 1H, J = 12.0–17.1 Hz, C**H** vinyl), 8.17 (dd, 1H, J = 11.6–17.4 Hz, C**H** vinyl), 9.46 (br s, 3H, **H**-*meso*), 9.34 (br s, 1H, **H**-*meso*). Anal. calcd for $C_{56}H_{86}N_{12}O_2$: C, 70.15; H, 9.04; N, 20.45. Found: C, 69.88; H, 8.91; N, 20.14. MS (MALDI) *m*/*z* 959.7 ([M+H$^+$] monoisotopic calcd: 958.7).

5.2.25. 13,17-di-[3-Amidoethyl-*N*^{4}-(4-aminobutyl)spermine]-2,7,12,18-tetramethyl-8,13-divinyl-porphyne (19). R_f = 0.60 (CH_3CN/H_2O 7:3 + 1% TFA). UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1} × 10^3): 402 (29.7); 503 (2.6); 538 (2.1); 575 (1.4); 629 (0.9). ^{1}H NMR (CD_3OD, 400.13 MHz): δ (ppm) = 1.45–1.26 (m, 24H, CONHCH$_2$ (C**H**$_2$)$_2$CH$_2$N(CH$_2$)$_2$, (CH$_2$)$_2$NCH$_2$C**H**$_2$C**H**$_2$CH$_2$NH-CH$_2$C**H**$_2$CH$_2$NH$_2$ and (CH$_2$)$_2$NCH$_2$C**H**$_2$CH$_2$ NH$_2$), 3.22-2.88 (m, 36H, (C**H**$_2$)$_3$N, –(C**H**$_2$)$_2$NH, CONHC**H**$_2$-, C**H**$_2$NH$_2$ and Proto-CH$_2$-C**H**$_2$-CO), 3.50 (m, 12H, C**H**$_3$ β pyrrole), 4.33 (m, 4H, Proto-C**H**$_2$-CH$_2$-CO), 6.36 (m, 4H, C**H**$_2$ vinyl), 8.33-8.17 (m, 2H, C**H** vinyl), 9.52–9.41 (s, 4H, **H**-*meso*). Anal. calcd for $C_{62}H_{100}N_{14}O_2$: C, 69.36; H, 9.39; N, 18.26. Found: C, 68.89; H, 9.01; N, 17.97. MS (MALDI) *m*/*z* 1073.9 ([M+H$^+$] monoisotopic calcd: 1073.5).

5.3. 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 (**16**–**19**) 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.[22]

5.4. Cell culture

K562 human chronic myelogenous leukemia cell line was grown in RPMI-1640 medium containing 10% fetal calf serum, 1% antibiotic (penicillin, streptomycin), and 1% L-glutamine. Cultures were kept under a fully humidified atmosphere composed of 95% air and 5% CO_2 at 37 °C. Cells were subcultured twice a week and maintained in exponential growth.

5.5. Cell irradiation and analysis

Two white bulbs (30 W each, output 400–800 nm) have been used, giving a light fluence of 10 mW/cm^2 (fluence measured with a Digital Lux tester 1065 [YFE]). Photocytotoxicity of **16**, **17**, **18**, and **19** was determined on K562 cells and compared to that of Photofrin II®. Before the treatment with porphyrin, cells were washed and resuspended in the culture medium. Cell count was adjusted to 5×10^5 cells/mL and 2 μL of either porphyrin (final concentrations : 2×10^{-6} M or 2×10^{-7} M) or Photofrin II® (final concentration 1.25 μg/mL) was added to 2 mL of cell suspension in culture plate wells; cells illuminated without porphyrin and cells kept in the dark in the presence of porphyrins were used as controls in each experiment. Cells were irradiated during 30, 60, 90, and 120 min (fluence rate = 10 mW/cm^2) and then kept in the dark in the incubator for an additional 24 h. The dead cell count was estimated by flow cytometry in the presence of propidium iodide (PI) at once after illumination and after a further 24 h incubation in the dark.[20,21] All the compounds tested did not present any cytotoxicity in the dark. Data shown in Figures 3 and 4 are always means of three independent experiments. Annexin V/Propidium iodide co-staining assays (Roche Annexin-V-FLUOS) were conducted according to the manufacturer's protocol.

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