Bioorganic & Medicinal Chemistry 17 (2009) 7647–7657

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis of tetraglucosyl- and tetrapolyamine–tetrabenzoporphyrin conjugates for an application in PDT

Florian Ménard [a], Vincent Sol [a,*], Cyril Ringot [a], Robert Granet [a], Sandra Alves [b], Caroline Le Morvan [a], Yves Queneau [c], Noboru Ono [d], Pierre Krausz [a]

[a] Université de Limoges, Laboratoire de Chimie des Substances Naturelles EA 1069, Faculté des Sciences et Techniques, 123 Av. Albert Thomas, 87060 Limoges, France
[b] Laboratoire de Chimie Structurale Organique et Biologique, Université Pierre et Marie Curie, CNRS UMR7613, 4 Place Jussieu 75252 Paris Cedex 05, France
[c] Laboratoire de Chimie Organique, ICBMS UMR 5246 CNRS, Université de Lyon 1, INSA Lyon, bât. Jules-Verne, 20 Av. Albert-Einstein, 69621 Villeurbanne Cedex, France
[d] Department of Chemistry, Faculty of Science, Ehime University, 2-5 Bunkyo-cho, Matsuyama 790-8577, Japan

ARTICLE INFO

Article history:
Received 29 May 2009
Revised 14 September 2009
Accepted 25 September 2009
Available online 29 September 2009

Keywords:
Tetrabenzoporphyrin
Polyamine
Isomaltulose
D-Glucose
PDT

ABSTRACT

This paper reports the synthesis and characterization of a new class of tetrabenzoporphyrins bearing glucosyl or polyamine units on *meso* positions to improve the targeting of cancer cells. Photocytotoxic activity of these photosensitizers was tested on cell lines HaCaT and MCF-7 and compared to Photofrin II®.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Photodynamic therapy (PDT) appears as an innovative technology that was recently accepted to treat a number of cancers.[1] This technique is based on the selective accumulation of photosensitizers, such as porphyrins, followed by irradiation of the affected area with visible light. Upon irradiation, the excited state of the photosensitizer generates singlet oxygen that induces cell damage and ultimately leads to cell death.[2] Photofrin II®, a mixture of condensed porphyrins derived from haematoporphyrin, was the first photosensitizer approved for clinical PDT.[3] Second-generation photosensitizers are synthetic molecules with a well defined structure. For example, *meta*-tetrahydroxyphenylchlorin (*m*-THPC or Foscan®) has been approved for the treatment of esophagus, lung and larynx cancers.[4] Benzoporphyrin derivative monoacid ring A (BPD-MA), better known as verteporfin (Visudyne®), is currently used for the treatment of age-related macular degeneration (AMD).[5] Nevertheless, most of these compounds are highly hydrophobic and suffer from several drawbacks such as a lack of selectivity toward tumor cells and their use frequently results in persistent photosensitization of the skin. In order to enhance the solubility of photosensitizers in aqueous solutions, as well as their affinity for cancer cells, several strategies have been developed. Accordingly, macrocycles have been conjugated to amino-acids or peptides,[6] nucleotides,[7] monoclonal antibodies,[8] nanoparticles,[9] or carbohydrates.[10] Recently, and in connection with our research program on photosensitizers and their use in PDT, we have developed the synthesis of different photosensitizers (porphyrins and chlorins) bearing polyamine or glucosyl (α-D- or β-D-glucopyranose form) units.[11] In this article, we have extended this strategy to the synthesis of new tetrabenzoporphyrin conjugates in the main to investigate the effect of carbohydrate and polyamine moieties for PDT application. Indeed, tetrabenzoporphyrins (TBP), comprising four benzene rings fused to a porphyrin macrocycle and no phenyl rings on *meso* positions, could appear as promising candidates since they absorb light in the red region of the visible spectrum (at λ >640 nm) and much more strongly than porphyrins (such as Photofrin®).[12] Furthermore, extended π-conjugated systems endow tetrabenzoporphyrins with high chemical stability albeit decreased solubility compared with the porphyrin macrocycle. By contrast, *meso*-substituted tetraaryltetrabenzoporphyrins Ar_4TBP (with four phenyl rings on *meso* positions) proved much more soluble in organics solvents. The improved solubility of the latter is probably due in part to their considerably nonplanar structure, which is the consequence of the steric crowding induced by the four *meso*-aryl substituents. Nevertheless, spare solubility of unsubstituted tetraaryltetrabenzoporphyrins in aqueous media

* Corresponding author. Tel.: +33 (0) 5 55 45 74 90; fax: +33 (0) 5 55 45 77 81.
E-mail address: vincent.sol@unilim.fr (V. Sol).

0968-0896/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2009.09.048

presents a major drawback in biological environments. With the aim to increase the water solubility of Ar_4TBP, introduction of sulfonic acid, carboxylic acid or nido-carborane functions have been realized.[13] Taking into account these data, we have chosen to synthesize tetrabenzoporphyrins bearing glucosyl or polyamine units. Photosensitizers with sugar moieties are known be easily soluble in water and, in addition, glycosyl substituents also increase plasmatic life time;[14] these conjugates could be specifically recognized by lectins expressed by tumor cells.[15] Recently, Hao et al. described the attachment of galactose or lactose units to porphyrins by click-chemistry.[16] The rationale that supports polyamine derivatization has been already discussed[17] and relies upon the preferential uptake of polyaminated compounds into rapidly growing cells.[18] Polyamines are also known to bind phosphate moieties of nucleic acids by charge interaction and hydrogen bonding;[19] as a consequence, polyamine-conjugated photosensitizers were shown to possess DNA photo-damaging ability.[20]

In this paper, we describe the synthesis of four new Ar_4TBP bearing osidic **5**, **12** (Schemes 1 and 2) or polyamine units **17**, **18** (Scheme 3), along with full experimental data concerning their characterization (^{1}H NMR, MALDI, absorption and fluorescence spectroscopies) and their in vitro photocytotoxic properties compared at once parent tetraryltetrabenzoporphyrin Ar_4TBP without substituents on aryl positions and Photofrin II®.

2. Chemistry

2.1. Synthesis

The first synthesis of tetrabenzoporphyrin was described by Helberger[21] and was further developed by Linstead and co-workers[22] The few tetraaryltetrabenzoporphyrin synthesis protocols found in the literature, are based on the total synthesis of tetrabenzoporphyrin from oxygen-sensitive isoindoles,[23] self-condensation of benzodipyrromethene to give a dibenzoporphyrin,[24] synthesis of (poly)butanoporphyrins followed by DDQ oxidation,[25] Diels–Alder reaction on pyrrolo[3,4,*b*]porphyrins or sulfolenoporphyrins,[26] intramolecular cyclization[27] or Diels–Alder reactions on vinylporphyrins.[28] These structures can also be prepared via the condensation of tetrahydroisoindole with aromatic aldehyde followed by DDQ oxidation,[29] a protocol which we have used in the present study.

Syntheses of the new conjugated compounds **5**, **12**, **17** and **18** are summarized in Schemes 1–3. Tetrahydroisoindole **1** was obtained from 1-nitrocyclohexene with ethyl isocyanoacetate by Barton–Zard reaction[30] which was then followed by a procedure using a simple one-pot deprotection–decarboxylation.[29] Then, in a routine procedure, compound **1**, without intermediate isolation and purification, was directly subjected to the subsequent Lindsey synthesis[31] with aromatic aldehyde **2** (Scheme 1). Glucosylaldehyde **2** was synthesised from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[32] and 4-hydroxybenzaldehyde as described in previous papers.[33] The resulting product was isolated by chromatographic column purification and porphyrin **3** appeared as a green powder in 36% yield. Aromatization of tetracyclohexenoporphyrin **3** into tetrabenzoporphyrin **4** required its conversion into metal complex Zn–**3**.[29] We chose zinc since its decomplexation required softer conditions than for other metals (Cu, Ni, Pd). In fact osidic units did not withstand the hard conditions of demetallation of the latter metals. Thus metal complex of glucosylporphyrin **Zn–3** was prepared by reacting free base porphyrin **3** with zinc acetate in appropriate solvent ($CHCl_3$/MeOH, 9:1). Reaction was considered complete when the Soret band of the dication at 476 nm vanished. Zinc porphyrin was recovered in 62% yield as a dark red solid. This reaction was followed by oxidative aromatization of tetracyclohexenoporphyrin by DDQ **Zn–3** leading to tetrabenzoporphyrin Zn–**4**. A chromatographic purification afforded **Zn–4** (37% yield) which appeared as a green powder. Metallo-benzoporphyrin **Zn–4** was demetalled by HCl (10 M) in CH_2Cl_2 and compound **4** was collected and purified by thin-layer chromatography (TLC) (76% yield) (Scheme 1). Removal of the acetate protective groups of compound **4** with 0.5 M sodium methoxide in MeOH/CH_2Cl_2 (8:2) gave tetraglucosyl-tetrabenzoporphyrin **5** in 86% yield (Scheme 1). Synthesis of compound **12** started from tetraphenylcarboxytetrabenzoporhyrin **6** synthesized by the Vinogradov method.[29] In a second step, 2,2′-(ethylenedioxy)-bis-ethylamine used as hydrophilic linker, was synthesised by Schneider method.[34] The synthesis of glucosyl-benzoporphyrin **8** was carried out by reaction of compound **6**, with *tert*-butyl-*N*-(2-[2-(2-aminoethoxy)ethoxy]ethyl)-carbamate **7**, in the presence of dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) in dimethylformamide (Scheme 2). Then, the protecting groups (Boc) were removed by standard methods in high yields with TFA in dichloromethane at room temperature. Finally, the expected benzoporphyrin **9** was obtained in a nearly quantitative yield. Reaction of benzoporphyrin **9** with triacetyl lactone **10**,[35] with an excess of DMAP in $CHCl_3$ gave glucosylporphyrin **11**, in 28% yield, after purification on silica gel PLC. The last step, removal of the acetate protective groups **11** by 0.5 M sodium methoxide in MeOH/CH_2Cl_2 (8:2) gave tetrabenzoporphyrin **12** in 82% yield.

Synthesis of tetraarylbenzoporphyrin-polyamines **17**, **18** require the selective protection of spermidine (Spd) and spermine (Spm). Different approaches to the selective protection of polyamines have been reported.[36] We chose Boc-protective group, which can be selectively removed by trifluoroacetic acid (TFA). Thus, spermine and spermidine analogues **13**, **14** (Scheme 3) were obtained in three steps, as described previously.[37] Under the same conditions as for benzoporphyrin **8**, compound **6** 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 DCC and HOBt in DMF (Scheme 3). After purification by TLC, protected conjugated tetraaryltetrabenzoporphyrins **15** and **16** were obtained in 39% and 58% yields, respectively. The expected compounds **17** and **18** were obtained after cleavage of protecting groups (Boc), in quantitative yields. Finally, tetraaryltetrabenzoporphyrin derivatives **17** and **18** were obtained in a nearly quantitative yield. Compounds **17** and **18** are freely soluble in MeOH/H_2O, but not completely in water.

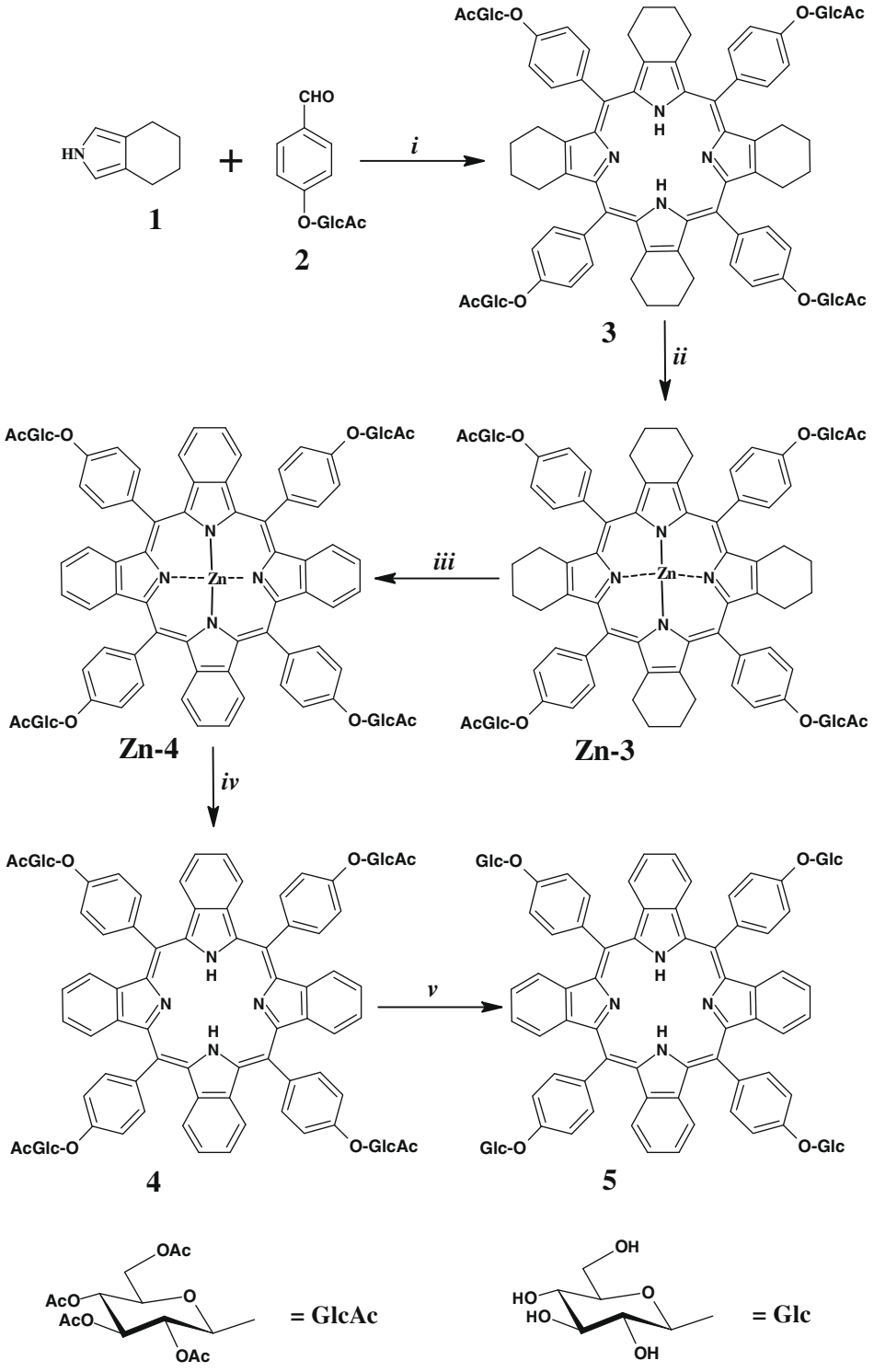
2.2. Mass characterization

Mass spectrometry of all porphyrin and Ar_4TBP derivatives was performed by using the matrix-assisted laser desorption ionization-time-of-flight (MALDI-TOF) technique. Most of the compounds studied gave one main peak (protonated molecule MH+, no fragments). Nevertheless, compounds **15**–**16** bearing Boc-protected polyamine units gave additional signals. The MALDI-TOF mass spectrum of compound **15** (Fig. 1) clearly indicated the presence of the protonated porphyrin derivative ($M+H^+$) along with metastable fragment ions that correspond to a loss of one or several protective groups.[37]

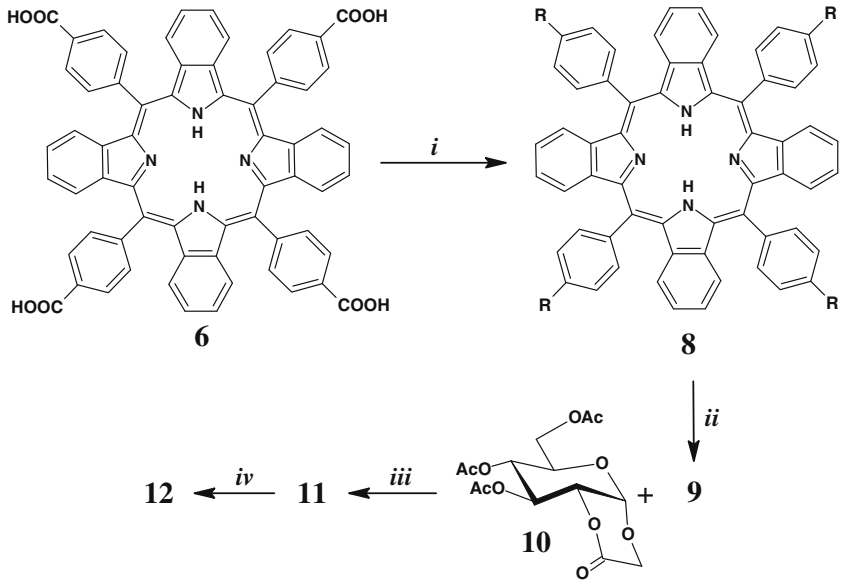
2.3. ^{1}H NMR characterization

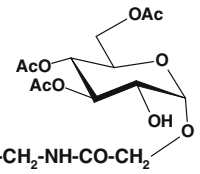
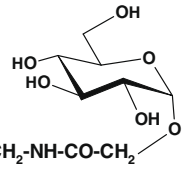
^{1}H NMR spectra recorded at 400.13 MHz were used for characterization of **3**, **4**, **8**, **14**, **15** and **16** in $CDCl_3$ or CD_3OD for deprotected compounds **5**, **9**, **12**, **17** and **18**. The detailed resonance assignments are based on integration and selective homonuclear decoupling and 2D homonuclear COSY experiments. The spectra of these compounds are governed by the symmetry properties of the molecule and by orientation of *meso* substituents (polyamines

with Boc-protected units in **8**, **15** and **16**, with free amino functions in **17–18**, the spacer arm in **9** and sugar moieties in **4**, **5**, **10** and **11**). ^{1}H NMR spectrum of benzoporphyrin derivative **11** (Fig. 2) is composed of seven groups: the phenyl protons appearing as doublet signals between 8.0 and 8.5 ppm, the aromatic ring protons appearing as doublet signals between 7.1 and 7.3 ppm, the spacer arm proton signal split into two sets near 3.4–3.9 ppm and 1.2–3.8 ppm, the glucosyl protons observed at 3.5–5.5 ppm, the acetate protective group protons near 2 ppm, and the NH protons appearing as a broadened singlet near −1.1 ppm. Integrations were in agreement with the fixation of four targeting units on the photosensitizers. Moreover, for glucosyl-benzoporphyrin **5**, the resonance of the four anomeric protons appeared as a doublet peak at 4.7 ppm. The high value of the coupling constant (J_{H-H} = 8.3 Hz) observed for these protons confirms that the sugar is in the β-D-glucopyranose conformation. The alpha configuration of glucosyl-benzoporphyrin **12** is confirmed by the low coupling constant (J_{H-H} = 3.5 Hz).



Scheme 1. Reagents and conditions: (i) $BF_3{\cdot}Et_2O$ (0.14 equiv), CH_2Cl_2, rt, 2 h, then DDQ (1.1 equiv), 1 h, 36%; (ii) $Zn(OAc)_2$, $CHCl_3$/MeOH (9:1), 1 h, 62%; (iii) DDQ (16 equiv), THF, reflux, 1 h, 37%; (iv) HCl (10 M), CH_2Cl_2, 5 min, 76%; (v) MeONa (0.5 M, 3 equiv per acetate group), CH_2Cl_2/MeOH (8:2), 1 h, 86%.



Benzoporphyrins	R
8	CO-NH-CH_2-(CH_2-O-CH_2)$_2$-CH_2-NHBoc
9	CO-NH-CH_2-(CH_2-O-CH_2)$_2$-CH_2-NH_2
11	CO-NH-CH_2-(CH_2-O-CH_2)$_2$-CH_2-NH-CO-CH_2 
12	CO-NH-CH_2-(CH_2-O-CH_2)$_2$-CH_2-NH-CO-CH_2 

Scheme 2. Reagents and conditions: (i) H_2N-CH_2-(CH_2-O-CH_2)$_2$-CH_2-NHBoc (**7**) (4.4 equiv), DCC (4.4 equiv), HOBt (4.4 equiv), DMF, rt, 85%; (ii) CF_3CO_2H/CH_2Cl_2 (1:1), rt, 2 h, quantitative yields; (iii) DMAP (16 equiv), $CHCl_3$, rt, 2 weeks, 28%; (iv) MeONa (0.5 M, 3 equiv per acetate group), CH_2Cl_2/MeOH (8:2), 30 min, 82%.

2.4. UV–vis absorption and fluorescence spectroscopies

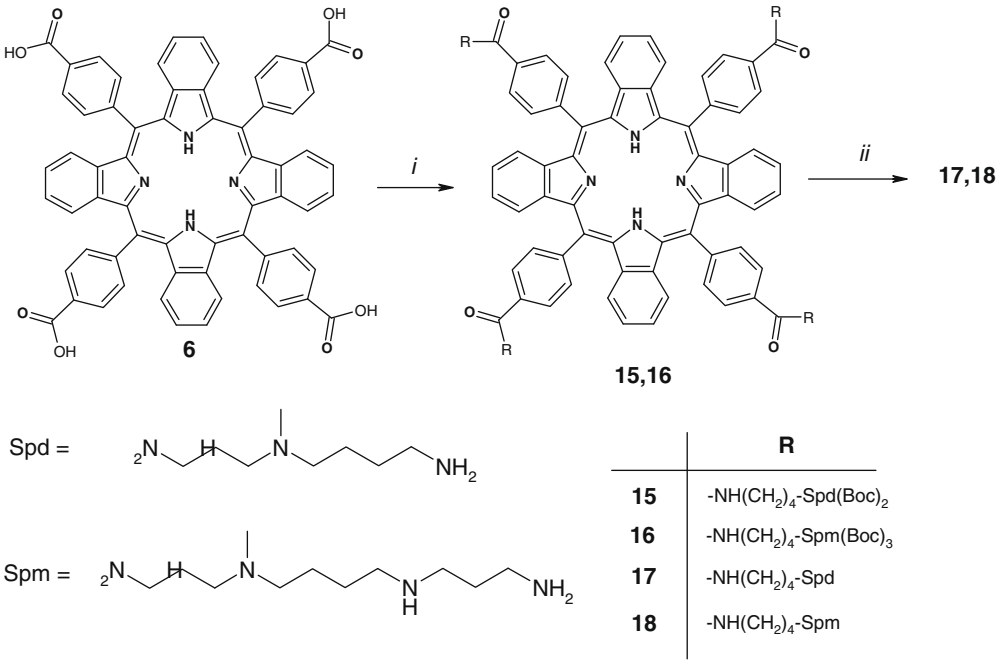
Spectra of the protected compounds (polyamino and polyglucosyl) were recorded in $CHCl_3$, and the deprotected compounds in MeOH and in water (Table 1). Tetraaryltetrabenzoporphyrin derivatives show typical spectra with a Soret band near 470 nm, and three less intense Q bands around 600, 645 and 700 nm, annotated QIII, QII and QI, respectively. Spectra of the final products are similar to spectra of related compounds and share a 645 nm band more intense than the corresponding band of porphyrins. Differences were observed in absorption spectra of the various protected or deprotected Ar_4TBP derivatives. Spectra of deprotected compounds **17** and **18** present a Soret band broader than protected compounds **15** and **16**. Furthermore, deprotected derivatized benzoporphyrins showed a blue shift and a drop in molar extinction coefficient compared to their protected counterparts. Compared with the literature,[38] all these features strongly suggest association of the deprotected benzoporphyrins in aqueous solvents. Thus, the low solubility of Ar_4TBP with polyamine moieties could be attributed to this association. Moreover, in the case of glucosylated Ar_4TBP with ethylenedioxy spacer arm between macrocycle and sugar units **12**, similar features were also observed.

All fluorescence spectra of dimers in MeOH were characterized by one or two emission bands ($655 < \lambda_{max} < 665$, for tetraaryltetrabenzoporphyrin derivatives **12**, **17**, **18** and 660 nm, 700 nm for **5**). The fluorescence emission wavelengths of **5**, **12**, **17** and **18** in aqueous solutions were identical to those obtained in MeOH (Table 2), but emission was strongly quenched. This decay of fluorescence can be explained by the formation of aggregates and is consistent with the features of the respective UV–vis spectra.[39] The low values of Φ_F for porphyrins **5**, **12**, **17** and **18** can be attributed to a partial aggregation of the photosensitizers in this medium.

3. Photophysical studies

3.1. Photostability of tetrabenzoporphyrin derivatives

In order to establish whether unprotected Ar_4TBP derivatives **5**, **12**, **17** and **18** could undergo photobleaching, photostability stud-



Scheme 3. Reagents and conditions: (i) polyamine **13** or **14** (4.4 equiv), DCC (4.4 equiv), HOBt (4.4 equiv), DMF, rt, 18 h, 39% (**15**), 58% (**16**); (ii) CF_3CO_2H/CH_2Cl_2 (1:1), rt, 2 h, quantitative yields for compounds **17** and **18**.

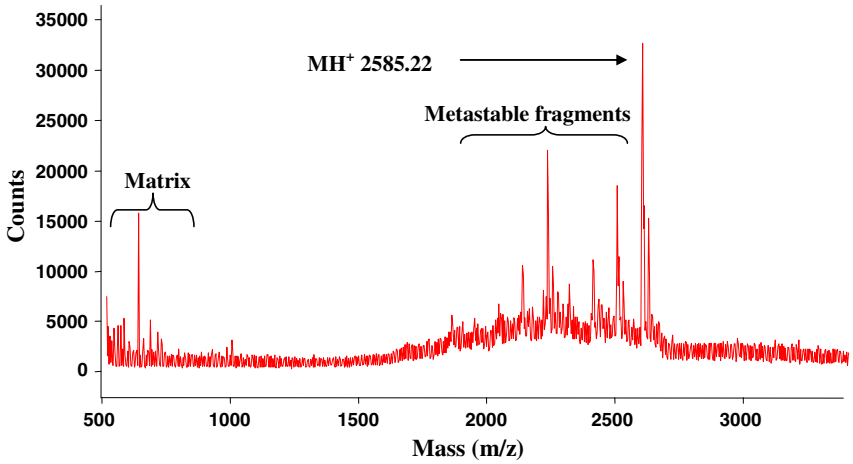


Figure 1. Example of MALDI mass spectra (compound **15**).

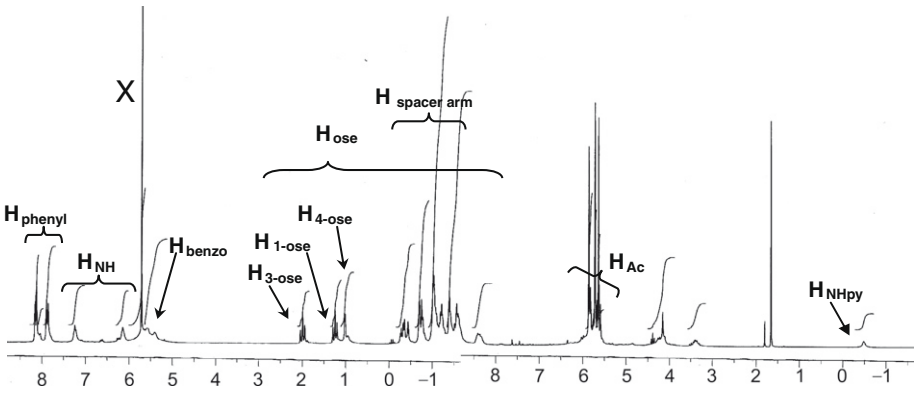


Figure 2. ^1H NMR in $CDCl_3$ of compound **11**.

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

Compound[a]	Soret	Visible bands (Q)
4(**b**)	466 (313.6)	591 (22.1), 642 (42.0), 697 (17.4)
5(**a**)	470 (299.6)	588 (23.3), 629 (35.2), 697 (14.7)
11(**b**)	469 (230.8)	596 (11.5), 648 (30.2), 701 (9.2)
12(**a**)	464 (99.7)	652 (12.2), 698 (1.7)
15(**b**)	467 (126.9)	603 (12.1), 648 (19.1), 699 (8.8)
16(**b**)	469 (173.8)	605 (10.8), 655 (21.6), 710 (9.2)
17(**a**)	462 (73.0)	594 (4.6), 639 (9.1), 696 (2.8)
18(**a**)	462 (55.5)	592 (5.7), 639 (8.1), 697 (4.5)

[a] Solvents as follows: (a) MeOH, (b) CH_2Cl_2.

Table 2
Fluorescence emission maximum (in MeOH) wavelength (λ_{max}), fluorescence (Φ_F) quantum yields and partition coefficient (log *P*)

Photosensitizers	λ_{max}	Φ_F	Log *P*
5	660/700	0.03	0.18
12	665	0.01	−0.75
17	655	0.01	−0.21
18	660	0.03	−0.47

ies were performed with white light (fluence rate 2.5 mW/cm^2). Results (Table 3) display the relative residual absorbance of samples in function of irradiation time.[40] 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

In order to determine the photosensitizing properties of Ar_4TBP derivatives **5**, **12**, **17** and **18**, trapping reactions of $^1O_2$ with ergosterol acetate were carried out.[41] Reference experiments with eosin, rose Bengal or haematoporphyrin (HP), known singlet oxygen producers, gave ergosterol acetate epidioxide with nearly quantitative yields. In the same experimental conditions, the benzoporphyrins described in this article were found as efficient as HP.

3.3. Partition coefficients

Lipophilicity has proven an important molecular descriptor that is often well-correlated with the bioactivity of drugs; log *P*, reflects the equilibrium partitioning of a molecule between a nonpolar and a polar phase, such as the 1-octanol/water system.[42] In this work, we have determined log *P* of tetraaryltetrabenzoporphyrin-tetraglucosylated derivatives **5**, **12**, and tetraaryltetrabenzoporphyrin tetrapolyamines derivatives **17** and **18** as log($[Ar_4TBP]_{1\text{-octanol}}$/$[Ar_4TBP]_{water}$) (Table 2).

Table 3
Photobleaching tests

Photosensitizers	Irradiation time (min)				
	0	**15**	**30**	**45**	**60**
5	100	100	99	98	95
12	100	100	100	98	97
17	100	100	99	97	96
18	100	100	100	99	97

Tetraaryltetrabenzoporphyrin solutions were irradiated (white light, fluence 2.5 mW/cm^2). Absorbance was measured at the maximum of the Soret band. Results represent residual absorbance (%) in function of irradiation time.

4. Biological assays

Glucosyl-tetraaryltetrabenzoporphyrin conjugates **5**, **12** and polyamine–tetraaryltetrabenzoporphyrin conjugates **17**, **18** were evaluated for their photocytotoxicity against human cell lines: HaCaT (keratinocyte cell line) and MCF-7 (breast adenocarcinoma cell line). Ar_4TBP[29a] without substituents under *meso*-aryl groups and Photofrin II® at the same concentration (this ponderal concentration was based on haematoporphyrin molecular weight of 600 g mol^{-1}) were used as references. This study aimed at evaluating benzoporphyrin derivative ability to induce cell death. IC_{50} of these photosensitizers were determined and compared to Photofrin II®. Results are presented in Table 4. All compounds were found less active than Photofrin II®. In particular, compounds **12**, **18** and Ar_4TBP were found inefficient as photosensitizers against HaCat and MCF-7 cell lines. Benzoporphyrins **5** and **17**, were more active against HaCat but displayed a very low activity against MCF-7. This low activity could be attributed to the strong hydrophilic character of each one of these compounds.[43]

5. Discussion

In this study, we describe the syntheses and biological evaluations of four new photosensitizers which differ by the nature of targeting units. In a first time, we have chosen to target cancer cells by the use of sugar units. So, two *meso*-tetraglycosylaryltetrabenzoporphyrins have been synthesized by attaching the osidic units directly on the macrocycle, or separated from the latter by a hydrophilic spacer. These compounds were obtained by different methods: the first one consisted in amidation followed by glycosylation of synthetic tetrabenzoporphyrin, and the second proceeded by condensation of glucosylated aldehydic precursors with tetrahydroisoindole. The latter approach opens up interesting prospects since the development of new functionalised asymmetric benzoporphyrins could not be obtained using commonly used methods. The second cellular recognition element employed in this study was polyamine units and to this end, two natural polyamines, spermidine and spermine, were grafted through an aminobutyl arm on tetracarboxyphenylbenzoporphyrin. Characterizations (MALDI, UV–vis, fluorescence and ^{1}H NMR) have confirmed the expected structures for these new protected and unprotected molecules. In addition, all these compounds showed a strong absorption band around 700 nm and displayed ability to produce singlet oxygen.

Influence of glucosyl units or polyamine units on the cellular viability has been evaluated. Preliminary tests to assess photocytotoxic activity of these molecules have shown that their activities were weaker than Photofrin II® used as a reference. Tetrakis benzoporphyrins **12**, **18** and Ar_4TBP (reference compound) were found inefficient as photosensitizers against HaCat and MCF-7 cell lines.

Table 4
IC_{50} (ng mL^{-1}) of tetraarylbenzoporphyrin derivatives **5**, **12**, **17**, **18**, tetraarylbenzoporphyrin Ar_4TBP and Photofrin® tested against HaCaT and MCF-7 human cell lines

Compounds	HACAT		MCF7	
	Mean	±ET	Mean	±ET
Photofrin®	78.7	39	1224.0	124
5	971.5	194	8414.6	2976
17	3417.8	161	7277.2	3915
12[a]	—	—	—	—
18[a]	—	—	—	—
Ar_4TBP[a,b]	—	—	—	—

[a] No significant result.
[b] This compound without sugar or polyamines units under phenyl ring was been synthesised by Vinogradov method[29a] and was been used as reference.

The other benzoporphyrin derivatives **5** and **17** were shown to be more active against HaCat but displayed a very low activity against MCF-7. In agreement with these preliminary results, we tried to correlate these different structures with PDT activities. The results obtained with compounds **12** and **18** could be attributable to the very strong hydrophilicity of these benzoporphyrins derivatives. Indeed, observations of UV–vis and fluorescence spectra have shown strong aggregation propensities. Furthermore, comparison of partition coefficients of tetraglucosylated benzoporphyrins derivatives **5** and **12** showed that tetraethylene glycol spacer arm increased hydrophilicity of compound **12** and thus decreased its PDT efficiency.[44] Moreover, inefficiency of Ar_4TBP, used as reference, showed that presence of four sugar units directly grafted on *meso* phenyl rings of tetraphenyltetrabenzoporphyrin derivative **5** was important for increase amphiphilic character of macrocycle and allowed evaluation of their photocytotoxicities against human cell lines (HaCat and MCF-7). Indeed, as described in the literature,[13,16] solubility in aqueous medium of tetraphenyltetrabenzoporphyrins without hydrophilic substituents (as sulfonic acid, nido-carborane or sugar units) was very lower and so, they were not used for biomedical application (PDT). Concerning the nature of polyamine group on the macrocycle, our results show that compound **18**, substituted by four spermine units, is less efficient than spermidine-substituted compound **17**, suggesting that the higher number of amine groups increases hydrophilicity of compound **18** and thus decreases PDT efficiency. New benzoporphyrin derivatives with modified hydrophilic/lipophilic balance are currently being designed with the aim to increase their amphiphilic character and thus their PDT efficiencies. For tetraglycosylbenzoporphyrin **5** which have shown some cytotoxic activity, studies are in progress to understand the mechanism of PDT-induced cell death and to track its cellular uptake and subcellular localization.

6. Conclusion

A series of new conjugated tetraglucosyl-tetrabenzoporphyrins **5**, **12** and tetrapolyamine–tetrabenzoporphyrins **17**, **18** have been designed, synthesized and characterized. In particular, the presence of glucosyl units directly grafted on *meso* phenyl ring (**5**), increased solubility in aqueous medium of tetraphenyltetrabenzoporphyrin derivatives. Thus, it was possible to realize preliminary viability tests, in vitro on two human cancer lines (MCF-7 and HaCaT) in presence of a polychromatic light and this photocytotoxicity activity is more important than for the parent compound Ar_4TBP without sugar units. Nevertheless first results showed that these compounds were less efficient than Photofrin®.

7. Experimental

7.1. General

All solvents and reagents were purchased from Aldrich, Prolabo or Acros. Triacetyl lactone **10** was supplied by Yves Queneau (Laboratoire de chimie organique—INSA Lyon). Analytical thin layer chromatography (TLC) was performed on Merck 60F254 or RP-18 F254S silica gel. Column chromatography was carried out with Merck silica gel (60 ACC; 15–40 μm). ^1H and ^{13}C NMR spectroscopies were performed with a Brüker DPX 400 spectrometer. Chemical shifts are reported as δ (ppm), downfield from internal TMS and are listed according to the standard numbering of tetraphenyltetrabenzoporphyrins and glucopyranose. UV–vis spectra were recorded on a Perkin–Elmer Lambda 25 double-beam spectrophotometer using 10- or 50-mm quartz cells. Fluorescence spectra were recorded on a PTI quanta master spectrofluorimeter equipped with a xenon short arc lamp (Ushio) and a photomultiplier tube (Hamamatsu R1527P). Fluorescence quantum yields have been determined using Rhodamine 101 as a standard. Corrections have been made to take into account the respective spectral response of the detection system. Infra-red spectra were recorded on a Perkin Elmer Spectrum 1000 FTIR spectrometer with KBr pellets. Elemental analyses were carried out by the 'Service Régional de Microanalyse de l'Université Pierre et Marie Curie, Paris'. 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) (Université Pierre et Marie Curie, Paris). It was operated in the reflection-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, were deposited onto the stainless steel sample slide and dried in air.

7.2. Synthesis

7.2.1. 5,10,15,20-Tetrakis-[4-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-tetracyclohexenoporphyrin 3

Compound **1** was synthesised by the Vinogradov method.[29] Isoindole **1** (0.15 g, 0.78 mmol, 1 equiv) was diluted with CH_2Cl_2 to about 100 mL. The vial was protected from light and flushed with Ar, and aromatic aldehyde **2** (0.36 g, 0.79 mmol, 1 equiv) was added in one portion. The solution was stirred for 10 min, then $BF_3{\cdot}Et_2O$ (13.5 μL, 0.11 mmol, 0.14 equiv) was added, and the mixture was stirred at rt for 2 h. DDQ (0.20 g, 0.85 mmol, 1.1 equiv) was added, and the mixture was left 1 h under continuous stirring. The resulting green solution was washed with 10% aq Na_2SO_3, with 10% aq Na_2CO_3, with 5% aq HCl, and finally with brine. The organic layer was dried over $MgSO_4$ and the solvent was evaporated in a vacuum. The chromatographic purification of the resulting solution on a silica gel column (eluent CH_2Cl_2/EtOH, 95:05 to 90:10) afforded **3** (0.16 g, 0.07 mmol) in 36% yield. Porphyrin **3** appeared as a green powder. $R_f = 0.52$ (CH_2Cl_2/EtOH, 95:05); UV–vis ($CHCl_3$): λ_{max}, nm (ε, L cm^{-1} mol$^{-1}{\cdot}10^{-3}$) 476 (134.9), 578 (8.6), 632 (10.3), 691 (19.1); ^1H NMR (400.13 MHz, $CDCl_3$, 25 °C) δ 8.02 (d, $J_{H-H} = 8.3$ Hz, 8H, $H_{3\text{-}5\text{-phenyl}}$), 7.32 (d, $J_{H-H} = 8.4$ Hz, 8H, $H_{2\text{-}6\text{-phenyl}}$), 5.25–5.50 (m, 16H, $H_{1''\text{-}2''\text{-}3''\text{-}4''}$), 4.49 (dd, $J_{H-H} = 4.5$ and $J_{H-H} = 12.4$ Hz, 4H, $H_{6''}$), 4.34 (dd, $J_{H-H} = 1.6$ and $J_{H-H} = 12.2$, 4H, $H_{6''}$), 4.28 (ddd, $J_{H-H} = 2.4$, $J_{H-H} = 5.4$ and $J_{H-H} = 10.7$, 4H, $H_{5''}$); 2.20 (m, 16H, $H_{1\text{-}4\text{-aryl}}$), 2.11 (s, 48H, H_{Ac}), 1.25–1.7 (m, 16H, $H_{2\text{-}3\text{-aryl}}$); MS (MALDI) *m/z* for $C_{116}H_{126}N_4O_{40}$, calcd 2214.79, found 2215.51 $[M+H]^+$.

7.2.2. Zinc-5,10,15,20-tetrakis-[4-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-tetracyclohexenoporphyrin Zn–3

An excess of $Zn(OAc)_2{\cdot}H_2O$ was added to a solution of porphyrin **3** (0.16 g, 70 μmol, 1 equiv) in 10 mL of $CHCl_3$/MeOH (9:1). The mixture was stirred until metal insertion was complete (after about 1 h), as evidenced by UV–vis spectroscopy. Reaction was considered complete when the Soret band of the dication at 476 nm vanished. The mixture was washed with 10% aq AcOH, with $NaHCO_3$, and with water and dried over $MgSO_4$. Solvent was evaporated in a vacuum and the remaining solid was purified on a silica gel column with use of a CH_2Cl_2/Et_3N (99:01) mixture as an eluent. Zn porphyrin (98 mg, 43 μmol) was recovered in 62% yield as dark red solid. **Zn–3** was identified by its UV–vis spectrum.

7.2.3. Zinc-5,10,15,20-tetrakis-[4-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-tetrabenzoporphyrin Zn–4

Metalloporphyrin **Zn–3** (98 mg, 43 μmol, 1 equiv) was dissolved in 150 mL of dry THF. A twofold excess of DDQ (0.16 g, 690 μmol, 16 equiv) was added, and the mixture was refluxed for 1 h. During refluxing, color changed from red-brown to deep green. The mixture was allowed to cool, diluted with CH_2Cl_2 (20 mL), washed with a 10% aq solution of K_2CO_3, with water and dried over $MgSO_4$. Solvent was removed in a vacuum, and the remaining solid was purified on a silica gel column with CH_2Cl_2/Et_3N (99:01) to CH_2Cl_2/EtOH/Et_3N (70:30:1). Chromatographic purification afforded **Zn–4** (36 mg, 16 μmol) in 37% yield. Benzoporphyrin **Zn–4** appeared as a green powder and was identified by its UV–vis spectrum.

7.2.4. 5,10,15,20-Tetrakis-[4-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-phenyl]-tetrabenzoporphyrin 4

HCl (10 m, 10 mL) was added to a stirred solution of metalloporphyrin **Zn–4** (36 mg, 16 μmol, 1 equiv) in 50 mL of CH_2Cl_2, which resulted in an immediate change of color from green to red-brown. The mixture was allowed to react for 5 min. The solution was transferred into a separatory funnel and washed with water, with a 10% aq solution of K_2CO_3 and with water again and dried over $MgSO_4$. The chromatographic purification of the resulting solution by thin-layer chromatography (CH_2Cl_2/MeOH, 92:08) afforded compound **4** (26.7 mg, 12 μmol) in 76% yield. R_f = 0.50 (CH_2Cl_2/EtOH, 97:03); UV–vis ($CHCl_3$): λ_{max}, nm (ε, L cm^{-1} mol^{-1}·10^{-3}) 466 (313.6), 591 (22.1), 642 (42.0), 697 (17.4); ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C) δ 8.28 (d, J_{H-H} = 8.4 Hz, 8H, $H_{3\text{-}5\text{-}phenyl}$), 7.50 (d, J_{H-H} = 8.4 Hz, 8H, $H_{2\text{-}6\text{-}phenyl}$), 7.30 (m, 16H, H_{benzo}), 5.50 (m, 12H, $H_{1''\text{-}2''\text{-}3''}$), 5.32 (t, J_{H-H} = 9.6 Hz, 4H, $H_{4''}$), 4.46 (dd, J_{H-H} = 5.2 and J_{H-H} = 12.3 Hz, 4H, $H_{6''}$), 4.29 (dd, J_{H-H} = 2.0 and J_{H-H} = 12.3 Hz, 4H, $H_{6''}$), 4.06 (ddd, J_{H-H} = 2.2, J_{H-H} = 5.2 and J_{H-H} = 9.7 Hz, 4H, $H_{5''}$), 2.17 (s, 48H, H_{Ac}), −1.18 (s_e, 2H, H_{NH}); MS (MALDI) *m/z* for $C_{116}H_{110}N_4O_{40}$, calcd 2199.7, found 2199.67 [M+H]$^+$. Microanal. Calcd for $C_{116}H_{110}N_4O_{40}$: C, 63.32; H, 5.04; N, 2.54. Found: C, 62.57; H, 4.22; N, 3.81.

7.2.5. 5,10,15,20-Tetrakis-[4-(β-D-glucopyranosyloxyphenyl)]-tetrabenzoporphyrin 5

Benzoporphyrin **4** (26.7 mg, 12 μmol, 1 equiv) was dissolved in CH_2Cl_2/MeOH (80:20, 5 mL), and sodium methoxide (0.5 M in MeOH, 3 equiv per acetate group) was then added. The mixture was stirred until benzoporphyrin **5** was totally precipitated (after about 1 h), and then filtered through a fritted disk, washed with MeOH and CH_2Cl_2. The resulting fine, green powder collected and purified by thin-layer chromatography ($CHCl_3$/EtOH, 9:1) afforded compound **5** (17.8 mg, 117 μmol) in 86% yield. R_f = 0.51 (MeOH/Et_3N, 99:01); UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1}·10^{-3}) 470 (299.6), 588 (23.3), 629 (35.2), 697 (14.7); ^{1}H NMR (400.13 MHz, CD_3OD, 25 °C) δ 8.33 (m, 8H, $H_{3\text{-}5\text{-}phenyl}$), 7.95 (m, 8H, $H_{2\text{-}6\text{-}phenyl}$), 7.1–7.3 (m, 16H, H_{benzo}), 4.69 (d, J_{H-H} = 8,3 Hz, 4H, $H_{1''}$), 4.4–4.6 (m, 20H, $H_{2''\text{-}3''\text{-}4''\text{-}6''\text{-}6''}$), 4.32 (m, 4H, $H_{5''}$); MS (MALDI) *m/z* for $C_{84}H_{78}N_4O_{24}$, calcd 1526.5, found 1527.0 [M+H]$^+$. Microanal. Calcd for $C_{84}H_{78}N_4O_{24}$·2H_2O: C, 64.52; H, 5.28; N, 3.58. Found: C, 63.37; H, 4.82; N, 4.51.

7.2.6. 5,10,15,20-Tetrakis-(4-[*tert*-butyl-*N*-(2-[2-(2-aminoethoxy)ethoxy]ethyl)-carbamate]-amidophenyl)-tetrabenzoporphyrin 8

tert-Butyl-*N*-(2-[2-(2-aminoethoxy)ethoxy]ethyl)-carbamate **7** (33 mg, 133 μmol, 4.4 equiv), synthesised by the Schneider method,[34] was dissolved in DMF. Carboxy-benzoporphyrin **6** (30 mg, 32 μmol, 1 equiv), synthesised by the Vinogradov method[29] was then added, followed by *N*,*N*′-dicyclohexylcarbodiimide (DCC) (27 mg, 131 μmol, 4.4 equiv) in dry DMF (1 mL). After addition of 1-hydroxybenzotriazole (HOBt) (18 mg, 133 μmol, 4.4 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 dichloromethane. The organic layer was washed with water (2 × 50 mL), dried over $MgSO_4$, and then evaporated to afford, after purification by thin-layer chromatography, the pure product. Compound **8** (46 mg, 24 μmol) was obtained in 85% yield. R_f = 0.32 ($CHCl_3$/EtOH/Et_3N, 70:30:01). UV–vis ($CHCl_3$): λ_{max}, nm (ε, L cm^{-1} mol^{-1}·10^{-3}) 469 (151.1), 596 (10.0), 646 (22.4), 703 (8.8); ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C) δ 8.47 (d, J_{H-H} = 7.0 Hz, 8H, $H_{3\text{-}5\text{-}phenyl}$), 8.33 (d, J = 6,96 Hz, 8H, $H_{2\text{-}6\text{-}phenyl}$), 7.20 (s, 8H, $H_{1\text{-}4\text{-}benzo}$), 7.13 (s, 8H, $H_{2\text{-}3\text{-}benzo}$), 6.54 (s, 4H, H_{NH}), 5.04 (s, 4H, H_{NH}), 3.88 (m, 16H, $H_{O\text{-}(CH2)2\text{-}O}$), 3.76 (m, 16H, $H_{CH2\text{-}O\text{-}(CH2)2\text{-}O\text{-}CH2}$), 3.62 (m, 8H, $H_{CO\text{-}NH\text{-}CH2}$), 3.36 (m, 8H, $H_{CH2\text{-}NH2}$), 1.39 (s, 36H, H_{CH3}), −1.09 (s_e, 2H, H_{NHpyr}); MS (MALDI) *m/z* for $C_{108}H_{126}N_{12}O_{20}$, calcd 1912.22, found 1912.30 [M+H]$^+$. Microanal. Calcd for $C_{108}H_{126}N_{12}O_{20}$·$H_2O$: C, 67.19; H, 6.68; N, 8.70. Found: C, 66.97; H, 6.43; N, 8.81.

7.2.7. 5,10,15,20-Tetrakis-(4-[*N*-(2-[2-(2-aminoethoxy)ethoxy]ethyl)-carbamate]-amidophenyl)-tetrabenzoporphyrin 9

The protecting groups (Boc) were removed by standard method in high yields with TFA in CH_2Cl_2 at room temperature (2 h). Benzoporphyrin **8** (46.2 mg, 24 μmol) reacted with TFA to afford pure product **9** (33.3 mg, 22 μmol). R_f = 0.69 (CH_3CN/H_2O/TFA 70:30:01). UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1}·10^{-3}) 469 (131.9), 595 (5.3), 645 (16.0), 700 (3.8); ^{1}H NMR (400.13 MHz, CD_3OD, 25 °C) δ 8.46 (d, J_{H-H} = 7.9 Hz, 8H, $H_{3\text{-}5\text{-}phenyl}$), 8.33 (d, J_{H-H} = 7.9 Hz, 8H, $H_{2\text{-}6\text{-}phenyl}$), 7.25 (m, 20H, H_{benzo} and H_{NH}), 3.82–3.90 (m, 16H, $H_{O\text{-}(CH2)2\text{-}O}$), 3.65–3.82 (m, 24H, $H_{(CH2)2\text{-}O\text{-}(CH2)2\text{-}O\text{-}CH2}$), 3.55–3.65 (m, 8H, $H_{CH2\text{-}NH2}$, 2.91 (br s, 8H, H_{NH2}), −1.09 (br s, 2H, H_{NHpyr}); MS (MALDI) *m/z* for $C_{88}H_{94}N_{12}O_{12}$, calcd 1511.8, found 1510.86 [M+H]$^+$. Microanal. Calcd for $C_{88}H_{94}N_{12}O_{12}$·2H_2O: C, 69.08; H, 6.45; N, 10.98. Found: C, 68.81; H, 6.23; N, 6.81.

7.2.8. 5,10,15,20-Tetrakis-[4-(3,4,6-tri-*O*-acetyl-α-D-glucopyranosyloxymethylcarbonyl-[*N*-(2-[2-(2-aminoethoxy)ethoxy]ethyl)])-carboxyphenyl]-tetrabenzoporphyrin 11

Benzoporphyrin **9** (33.3 mg, 22 μmol, 1 equiv) and triacetyl lactone **10** (40.4 mg, 116 μmol, 5.3 equiv), synthesised by Y. Queneau,[35] were diluted with $CHCl_3$ to about 30 mL, and DMAP (42 mg, 344 μmol, 16 equiv) was added in one portion. The mixture was stirred at rt for two weeks. The resulting green solution was evaporated in a vacuum and the chromatographic purification of the resulting solution on a silica gel column (eluent $CHCl_3$/EtOH, 85:15) afforded **11** (22 mg, 7.6 μmol) in 28% yield. R_f = 0.51 ($CHCl_3$/EtOH, 90:10); UV–vis ($CHCl_3$): λ_{max}, nm (ε, L cm^{-1} mol^{-1}·10^{-3}) 469 (230.8), 596 (11.5), 648 (30.2), 701 (9.2); ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C) δ 8.47 (d, J_{H-H} = 7.9 Hz, 8H, $H_{3\text{-}5\text{-}phenyl}$), 8.34 (d, J_{H-H} = 8.0 Hz, 8H, $H_{2\text{-}6\text{-}phenyl}$), 8.00 (m, 4H, H_{NH}),7.47 (m, 4H, H_{NH}), 7.19 (m, 8H, $H_{1\text{-}4\text{-}benzo}$), 7.11 (m, 8H, $H_{2\text{-}3\text{-}benzo}$), 5.39 (t, J_{H-H} = 9,6 Hz, 4H, $H_{3''}$), 5.03 (t, J_{H-H} = 9.8 Hz, 4H, $H_{4''}$), 4.91 (d, J_{H-H} = 3,5 Hz, 4H, $H_{1''}$), 4.17–4.28 (m, 8H, $H_{6'}$ and $H_{CH2\text{-}O\text{-}Ose}$), 3.99–4.05 (m, 8H, $H_{5''\text{-}6''}$), 3.55–3.90 (m, 56, $H_{2''}$, $H_{CH2\text{-}O\text{-}Ose}$ and $H_{(CH2)2\text{-}O\text{-}(CH2)2\text{-}O\text{-}(CH2)2}$), 2.10 (s, 12H, H_{Ac}), 2.03 (s, 12H, H_{Ac}), 1.99 (s, 12H, H_{Ac}), −1.07 (br s, 2H, H_{NHpyr}); MS (MALDI) *m/z* for $C_{144}H_{166}N_{12}O_{52}$, calcd 2896.9, found 2896.55 [M+H]$^+$. Microanal. Calcd for $C_{144}H_{166}N_{12}O_{52}$·$H_2O$: C, 59.33; H, 5.80; N, 5.76. Found: C, 59.01; H, 5.63; N, 5.81.

7.2.9. 5,10,15,20-Tetrakis-[4-(α-D-glucopyranosyloxymethylcarbonyl-[*N*-(2-[2-(2-aminoethoxy)ethoxy]ethyl)])-carboxyphenyl]-tetrabenzoporphyrin 12

Benzoporphyrin **11** (22 mg, 7.6 μmol, 1 equiv) was dissolved in CH_2Cl_2/MeOH (80:20, 2 mL), and sodium methoxide (0.5 M in MeOH, 3 equiv per acetate group) was then added. The mixture

was stirred until benzoporphyrin **12** was completely precipitated (after about 30 min), and then filtered through a fritted disk, washed with MeOH and CH_2Cl_2. The resulting fine, green powder was collected and purified by thin-layer chromatography ($CHCl_3$/EtOH, 9:1) and afforded compound **12** (18 mg, 6.2 μmol) in 82% yield. R_f (reverse-TLC) = 0.75 (MeOH); UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1}·10^{-3}) 464 (99.7), 652 (12.2), 698 (1.7); ^{1}H NMR (400.13 MHz, CD_3OD, 25 °C) δ 8.30 (br s, 16H, H_{phenyl}), 6.80–7.30 (m, 24H, H_{benzo} and H_{NH}), 4.84 (m, 4H, $H_{1''}$), 4.13 (m, 4H, $H_{5''}$), 3.9 (m, 4H, $H_{6''}$), 3.45–3.90 (m, 72H, $H_{2''\text{-}3''\text{-}4''\text{-}6''}$, $H_{CH2\text{-}O\text{-}Ose}$ and $H_{(CH2)2\text{-}O\text{-}(CH2)2\text{-}O\text{-}(CH2)2}$); MS (MALDI) *m/z* for $C_{120}H_{142}N_{12}O_{40}$, calcd 2392.4, found 2392.67 [M+H]$^+$. Microanal. Calcd for $C_{120}H_{142}N_{12}O_{40}\cdot 3H_2O$: C, 58.91; H, 6.10; N, 6.87. Found: C, 58.65; H, 5.97; N, 7.01.

7.2.10. General procedure for the synthesis of benzoporphyrins bearing spermine or spermidine units

N ^{4}-(4-Aminobutyl)-*N*^{1},*N*^{8}-bis-*tert*-butoxycarbonylspermidine **13**[37] (1.1 equiv per carboxy group), or *N*^{4}-(4-aminobutyl)-*N*^{1},*N*^{8},*N*^{12}-tris-*tert*-butoxycarbonylspermine **14**[37] (1.1 equiv per carboxy group) were dissolved in DMF. A solution of carboxy-benzoporphyrin **6** (1 equiv), was synthesised by the Vinogradov method, and *N*,*N*′-dicyclohexylcarbodiimide (DCC) (4.4 equiv) in dry DMF (3 mL) was added. After addition of 1-hydroxybenzotriazole (HOBt) (4.4 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 CH_2Cl_2. The organic layer was washed with water (2 × 50 mL), dried over $MgSO_4$, and then evaporated to afford, after purification by thin-layer chromatography, the pure product.

7.2.11. 5,10,15,20-Tetrakis-(*N*^{1},*N*^{8}-bis-*tert*-butoxycarbonyl spermidine-(*N*^{4}-(4-aminobutyl)-4-amidophenyl))-tetrabenzoporphyrin 15

Benzoporphyrin **6** (11 mg, 14.4 μmol, 1 equiv) and compound **13** (35 mg, 87 μmol, 6 equiv) reacted with DCC (10.3 mg, 50 μmol, 4.4 equiv) and 1-hydroxybenzotriazole (HOBt) (6.8 mg, 50 μmol, 4.4 equiv) to afford pure product **15** (15.0 mg, 5.6 μmol) in 39% yield. R_f = 0.35 ($CHCl_3$/EtOH/Et_3N, 70:30:01). UV–vis ($CHCl_3$): λ_{max}, nm (ε, L cm^{-1} mol^{-1}·10^{-3}) 467 (126.9), 603 (12.1), 648 (19.1), 699 (8.8); ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C) δ 8.41 (m, 16H, H_{phenyl}), 7.26 (s, 16H, H_{benzo}), 7.04 (br s, 4H, $H_{CO\text{-}NH}$), 5.32 (br s, 4H, H_{NHBoc}), 4.85 (br s, 4H, H_{NHBoc}), 3.75 (m, 8H, $H_{CO\text{-}NH\text{-}CH2}$), 3.70 (m, 8H, $H_{N\text{-}(CH2)3\text{-}CH2\text{-}NHBoc}$), 3.62 (m, 8H, $H_{N\text{-}(CH2)2\text{-}CH2\text{-}NHBoc}$), 3.15–3.23 (m, 24H, $H_{CO\text{-}NH\text{-}(CH2)3\text{-}CH2}$, $H_{N\text{-}CH2\text{-}(CH2)2\text{-}NHBoc}$ and $H_{N\text{-}CH2\text{-}(CH2)3\text{-}NHBoc}$), 1.75–1.95 (m, 40H, $H_{CO\text{-}NH\text{-}CH2\text{-}(CH2)2}$, $H_{N\text{-}CH2\text{-}(CH2)\text{-}CH2\text{-}NHBoc}$ and $H_{N\text{-}CH2\text{-}(CH2)2\text{-}CH2\text{-}NHBoc}$), 1.42 (m, 72, H_{CH3}), −1.1 (br s, 2H, H_{NHpyr}); MS (MALDI) *m/z* for $C_{148}H_{206}N_{20}O_{20}$, calcd 2585.34, found 2585.22 [M+H]$^+$. Microanal. Calcd for $C_{148}H_{206}N_{20}O_{20}\cdot H_2O$: C, 68.27; H, 7.98; N, 10.75. Found: C, 67.97; H, 7.89; N, 10.81.

7.2.12. 5,10,15,20-Tetrakis-(*N*^{1},*N*^{8},*N*^{12}-tris-*tert*-butoxycarbonyl spermine-(*N*^{4}-(4-aminobutyl)-4-amidophenyl))-tetra-benzoporphyrin 16

Benzoporphyrin **6** (11 mg, 14.4 μmol, 1 equiv) and compound **14** (49 mg, 87 μmol, 6 equiv) reacted with DCC (10.3 mg, 50 μmol, 4.4 equiv) and 1-hydroxybenzotriazole (HOBt) (6.8 mg, 50 μmol, 4.4 equiv) to afford pure product **16** (27.0 mg, 8.4 μmol) in 58% yield. R_f = 0.34 ($CHCl_3$/EtOH/Et_3N, 70:30:01). UV–vis ($CHCl_3$): λ_{max}, nm (ε, L cm^{-1} mol^{-1}·10^{-3}) 469 (173.8), 605 (10.8), 655 (21.6), 710 (9.2); ^{1}H NMR (400.13 MHz, $CDCl_3$, 25 °C) δ 8.44 (m, 16H, H_{phenyl}), 7.26 (s, 16H, H_{benzo}), 7.19 (br s, 4H, $H_{CO\text{-}NH}$), 5.29 (s, 12H, H_{NHBoc}), 3.60–3.80 (m, 40H, $H_{CO\text{-}NH\text{-}CH2}$, $H_{N\text{-}(CH2)2\text{-}CH2\text{-}NHBoc}$ and $H_{N\text{-}(CH2)3\text{-}CH2\text{-}NBoc\text{-}CH2\text{-}CH2\text{-}CH2\text{-}NHBoc}$), 3.18–3.25 (m, 24H, $H_{CO\text{-}NH\text{-}(CH2)3\text{-}CH2}$, $H_{N\text{-}CH2\text{-}(CH2)2\text{-}NHBoc}$ and $H_{N\text{-}CH2\text{-}(CH2)3\text{-}NHBoc}$), 1.60–1.70 (m, 48H, $H_{CO\text{-}NH\text{-}CH2\text{-}(CH2)2}$, $H_{N\text{-}CH2\text{-}(CH2)\text{-}CH2\text{-}NHBoc}$ and $H_{N\text{-}CH2\text{-}(CH2)2\text{-}CH2\text{-}NBoc\text{-}CH2\text{-}CH2\text{-}CH2\text{-}NHBoc}$), 1.43 (m, 108H, H_{CH3}), −1.1 (br s, H_{NHpyr}); MS (MALDI) *m/z* for $C_{180}H_{266}N_{24}O_{28}$, calcd 3214.18, found 3217.86 [M+H]$^+$. Microanal. Calcd for $C_{180}H_{266}N_{24}O_{28}\cdot H_2O$: C, 66.88; H, 8.36; N, 10.40. Found: C, 66.57; H, 8.19; N, 10.61.

7.2.13. General procedure for removal of Boc-protective groups

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

7.2.14. 5,10,15,20-Tetrakis-(spermidine-(*N*^{4}-(4-aminobutyl)-4-amidophenyl))-tetrabenzoporphyrin 17

Benzoporphyrin **15** (15 mg, 5.6 μmol) reacted with TFA to afford pure product **17** (8.8 mg, 4.9 μmol). R_f = 0.46 (CH_3CN/H_2O/TFA 70:30:0.5). UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1}·10^{-3}) 462 (73.0), 594 (4.6), 639 (9.1), 696 (2.8); ^{1}H NMR(400.13 MHz, CD_3OD, 25 °C) δ 8.46 (m, 8H, $H_{3\text{-}5\text{-}phenyl}$), 8.40 (m, 8H, $H_{2\text{-}4\text{-}phenyl}$), 7.22 (s, 8H, $H_{1\text{-}4\text{-}benzo}$), 7.12 (s, 8H, $H_{2\text{-}3\text{-}benzo}$), 3.50–3.0 (m, 24H, $H_{CO\text{-}NH\text{-}CH2}$, $H_{N\text{-}(CH2)2\text{-}CH2\text{-}NH2}$ and $H_{N\text{-}(CH2)3\text{-}CH2\text{-}NH2}$), 2.50–3.10 (m, 24H, $H_{CO\text{-}NH\text{-}(CH2)3\text{-}CH2}$, $H_{N\text{-}CH2\text{-}(CH2)2\text{-}NHBoc}$ and $H_{N\text{-}CH2\text{-}(CH2)3\text{-}NHBoc}$), 1.18–1.47 (m, 40H, $H_{CO\text{-}NH\text{-}CH2\text{-}(CH2)2}$, $H_{N\text{-}CH2\text{-}(CH2)\text{-}CH2\text{-}NHBoc}$ and $H_{N\text{-}CH2\text{-}(CH2)2\text{-}CH2\text{-}NHBoc}$); MS (MALDI) *m/z* for $C_{108}H_{142}N_{20}O_4$, calcd 1784.40, found 1784,72 [M+H]$^+$. Microanal. Calcd for $C_{108}H_{142}N_{20}O_4\cdot 2H_2O$: C, 71.96; H, 7.94; N, 15.54. Found: C, 71.52; H, 7.79; N, 15.78.

7.2.15. 5,10,15,20-Tetrakis-(spermine-(*N*^{4}-(4-aminobutyl)-4-amidophenyl))-tetrabenzoporphyrin 18

Benzoporphyrin **16** (27 mg, 8.4 μmol) reacted with TFA to afford pure product **18** (15.7 mg, 7.8 μmol). R_f = 0.69 (CH_3CN/H_2O/TFA 70:30:0.5). UV–vis (MeOH): λ_{max}, nm (ε, L cm^{-1} mol^{-1}·10^{-3}) 462 (55.5), 592 (5.7), 639 (8.1), 697 (4.5); ^{1}H NMR (400.13 MHz, CD_3OD, 25 °C) δ 8.40–8.48 (m, 16H, H_{phenyl}), 7.40 (s_e, 8H, $H_{1\text{-}4\text{-}benzo}$), 7.19 (br s, 8H, $H_{2\text{-}3\text{-}benzo}$), 3.50–3.70 (m, 40H, $H_{CO\text{-}NH\text{-}CH2}$, $H_{N\text{-}(CH2)2\text{-}CH2\text{-}NH2}$ and $H_{N\text{-}(CH2)3\text{-}CH2\text{-}NH\text{-}CH2\text{-}CH2\text{-}CH2\text{-}NH2}$), 2.50–3.10 (m, 24H, $H_{CO\text{-}NH\text{-}(CH2)3\text{-}CH2}$, $H_{N\text{-}CH2\text{-}(CH2)2\text{-}NH2}$ and $H_{N\text{-}CH2\text{-}(CH2)3\text{-}NH2}$), 1.50–1.90 (m, 48H, $H_{CO\text{-}NH\text{-}CH2\text{-}(CH2)2}$, $H_{N\text{-}CH2\text{-}(CH2)\text{-}CH2\text{-}NH2}$ and $H_{N\text{-}CH2\text{-}(CH2)2\text{-}CH2\text{-}NH\text{-}CH2\text{-}CH2\text{-}CH2\text{-}NHBoc}$); MS (MALDI) *m/z* for $C_{120}H_{170}N_{24}O_4$, calcd 2012.79, found 2012,13 [M+H]$^+$. Microanal. Calcd for $C_{120}H_{170}N_{24}O_4\cdot 2H_2O$: C, 70.96; H, 8.43; N, 16.55. Found: C, 70.57; H, 8.22; N, 16.81.

7.3. Singlet oxygen production

Photosensitizers (10^{-5} M) and ergosterol acetate were dissolved in DMF. Mixtures were illuminated during 30 min with two white bulbs (30 W each, output 400–800 nm) giving a fluence of 10 mW/cm^2, under oxygen atmosphere and at room temperature. Then, the appearance of ergosterol acetate epidioxide (EEP) was monitored by TLC (R_f = 0.3 eluent $CHCl_3$).

7.4. 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 (**5**, **12**, **17** and **18**) 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.

7.5. Cell culture

Cell cultures were kept under a fully humidified atmosphere composed of 95% air and 5% CO_2 at 37 °C. The tests were conducted on two cell lines HaCaT and MCF-7. HaCaT cell line was grown in 'Keratinocyte Serum-Free Medium' (KSFM, Invitrogen cell culture) supplemented with bovine pituitary extract (BPE) and recombi-

nant epidermal growth factor (rEGF). MCF-7 cell line was grown in 'Eagle's Minimum Essential Medium' supplemented with Earls salts (MEM) and 2 mM L-glutamine, 0.1 mM of nonessential amino acids (NeAA), 10% fetal calf serum (FCS) and 10 mM sodium pyruvate. Cultures, grown in presence of 1% antibiotics (penicillin, streptomycin) were subcultured twice a week and maintained in exponential growth.

7.6. Cell irradiation and analysis

Photocytotoxicity of **5**, **12**, **17** and **18** was determined on HaCat and MCF-7 cells and compared to that of Ar_4TBP and Photofrin II®. Cells were resuspended in the culture medium and adjusted to 5×10^5 cells/mL. After 48 h and medium removal by aspiration, 100 μL/well of either benzoporphyrin derivatives or Photofrin II® (final concentrations: 9×10^{-6}–10^{-2} mg/mL) were added to adherent cells in culture plate. The ponderal concentration of Photofrin II ® was based on haematoporphyrin molecular weight (600 Da). After 24 h of culture in the presence of photosensitizers, the culture medium was replaced by sterile PBS (100 μL/well). Cells were illuminated for 2 h with white bulbs giving a fluence rate of 5.5×10^{-2} mW/cm^2. PBS was then replaced by culture medium (100 μL/well) and the cultures incubated for an additional 24 h. Cells illuminated without photosensitizer and cells kept in the dark in presence of benzoporphyrins were used as controls in each experiment. Then, each well received 15 μL MTT reagent and cultures were incubated for 3 h at 37 °C in presence of 5% CO_2. Reaction was stopped by the addition of 100 μL 'stop' solution (DMSO). Absorbance was read at 595 nm and IC_{50} (50% Inhibitory Concentration) was determined for each molecule. IC 50 values were calculated as previously described by Banfi et al.[45]

Acknowledgments

We thank 'Conseil Régional du Limousin' for financial support and Dr. Michel Guilloton for critical reading of this manuscript.

References and notes

1. (a) Ortner, M. *J. Hepatobiliary Pancreat. Surg.* **2001**, *8*, 137; (b) Zoepf, T.; Jakobs, R.; Arnold, J. C.; Apel, D.; Rosenbaum, A.; Riemann, J. F. *Am. J. Gastroenterol.* **2001**, *96*, 2093; (c) Krishnamurthy, S.; Powers, S. K.; Witmer, P.; Brown, T. *Lasers Surg. Med.* **2000**, *27*, 224; (d) Kostron, H.; Obwegeser, A.; Jakober, R. *J. Photochem. Photobiol., B* **1996**, *36*, 157.
2. (a) Moan, J.; Peng, Q. *Anticancer Res.* **2003**, *23*, 3591; (b) MacDonald, I. J.; Dougherty, T. J. *J. Porphyrins Phthalocyanines* **2001**, *5*, 105; (c) Kessel, D. *Photodiag. Photodynam. Ther.* **2004**, *1*, 3.
3. Dougherty, T. J.; Potter, W. R.; Weishaupt, K. R. *Prog. Clin. Biol. Res.* **1984**, *170*, 301.
4. (a) Bonnett, R.; Djelal, B. D.; Nguyen, A. *J. Porphyrins Phthalocyanines* **2001**, *5*, 652; (b) Nathan, T. R.; Whitelaw, D. E.; Chang, S. C.; Lees, W. R.; Ripley, P. M.; Payne, H.; Parkinson, M. C.; Emberton, M.; Gilliams, A. R.; Mundy, A. R.; Bown, S. G. *J. Urol.* **2002**, *168*, 1427.
5. (a) Soubrane, G.; Bressler, N. M. *Br. J. Ophthalmol.* **2001**, *85*, 483; (b) Messmer, K. J.; Abel, S. R. *Ann. Pharmacother.* **2001**, *35*, 1593.
6. (a) Sol, V.; Blais, J. C.; Bolbach, G.; Carré, V.; Granet, R.; Guilloton, M.; Spiro, M.; Krausz, P. *Tetrahedron Lett.* **1997**, *38*, 6391; (b) Chaleix, V.; Sol, V.; Huang, H. M.; Guilloton, M.; Granet, R.; Blais, J. C.; Krausz, P. *Eur. J. Org. Chem.* **2003**, *1*, 1486; (c) Chaleix, V.; Sol, V.; Guilloton, M.; Granet, R.; Krausz, P. *Tetrahedron Lett.* **2004**, *45*, 5295; (d) Sibrian-Vasquez, M.; Jensen, T. J.; Hammer, R. P.; Vicente, M. G. H. *J. Med. Chem.* **2006**, *49*, 1364; (e) Schneider, R.; Tirand, L.; Frochot, C.; Vanderesse, R.; Thomas, N.; Gravier, J.; Guillemin, F.; Barberi-Heyob, M. *Anti-Cancer Agents Med. Chem.* **2006**, *6*, 469.
7. (a) Mestre, B.; Pitié, M.; Loup, C.; Claparols, C.; Pratviel, G.; Meunier, B. *Nucleic Acids Res.* **1997**, *25*, 1022; (b) Li, H.; Federova, O. S.; Trumble, W. R.; Fletcher, T. R.; Czuchajowski, L. *Bioconjugate Chem.* **1997**, *8*, 49; (c) Mammana, A.; Asakawa, T.; Bitsch-Jensen, K.; Wolfe, A.; Chaturantabut, S.; Otani, Y.; Li, X.; Li, Z.; Nakanishi, K.; Balaz, M.; Ellestad, G. A.; Berova, N. *Bioorg. Med. Chem.* **2008**, *16*, 6544.
8. Hudson, R.; Carcenac, M.; Smith, K.; Madden, L.; Clarke, O. J.; Pèlegrin, A.; Greenman, J.; Boyle, R. W. *Br. J. Cancer* **2005**, *92*, 1442.
9. (a) Lucas, R.; Granet, R.; Sol, V.; Le Morvan, C.; Policar, C.; Rivière, E.; Krausz, P. *e-Polymers* **2007**, *89*; (b) Brevet, D.; Gary-Bobo, M.; Raehm, L.; Richeter, S.; Hocine, O.; Amro, K.; Loock, B.; Couleaud, P.; Frochot, C.; Morère, A.; Maillard, P.; Garcia, M.; Durand, J. O. *Chem. Commun.* **2009**, *12*, 1475; (c) Chen, Y.; Samia, A. C.; Meyers, J. D.; Panagopoulos, I.; Fei, B.; Burda, C. *J. Am. Chem. Soc.* **2008**, *32*, 10643.
10. (a) Momenteau, M.; Maillard, P.; De Bélinay, M.-A.; Carrez, D.; Croisy, A. *J. Biomed. Opt.* **1999**, *4*, 298; (b) Schell, C.; Hombrecher, H. K. *Chem. Eur. J.* **1999**, 587; (c) Laville, I.; Figueiredo, T.; Loock, B.; Pigaglio, S.; Maillard, P.; Grierson, D. S.; Carrez, D.; Croisy, A.; Blais, J. *Bioorg. Med. Chem.* **2003**, *11*, 1643; (d) Li, G.; Pandey, S. K.; Graham, A.; Dobhal, M. P.; Mehta, R.; Chen, Y.; Gryshuk, A.; Rittenhouse-Olson, K.; Oseroff, A.; Pandey, R. K. *J. Org. Chem.* **2003**, *69*, 158; (e) Frochot, C.; Di Stasio, B.; Barberi-Heyob, M.; Carré, M. C.; Zwier, J. M.; Guillemin, F.; Viriot, M. L. *Oftalmologia* **2003**, *56*, 62; (f) Zhang, X.; Pandey, R. K. *Anti-Cancer Agents Med. Chem.* **2008**, *8*, 241.
11. (a) Lamarche, F.; Sol, V.; Huang, Y. M.; Granet, R.; Guilloton, M.; Krausz, P. *J. Porphyrins Phthalocyanines* **2002**, *6*, 130; (b) Garcia, G.; Sol, V.; Lamarche, F.; Granet, R.; Guilloton, M.; Champavier, Y.; Krausz, P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3188; (c) Sol, V.; Charmot, A.; Krausz, P.; Trombotto, S.; Queneau, Y. *J. Carbohydr. Chem.* **2006**, *25*, 345.
12. (a) Lash, T. D. In *The Porphyrin Handbook*; Kadish, K. M., Smith, K. M., Guilard, R., Eds.; Academic: New York, 2000; p Chapter 10; (b) Bonnet, R. In *Chemical Aspect of Photodynamic Therapy; Advanced Chemistry Texts*; Gordon and Breach Science: Amsterdam, 2000; (c) Lash, T. D. *J. Porphyrins Phthalocyanines* **2001**, *5*, 267; (d) Graça, M.; Vicente, H.; Smith, K. M. *J. Porphyrins Phthalocyanines* **2004**, *8*, 26.
13. (a) Vinogradov, S. A.; Wilson, D. F. *J. Chem. Soc., Perkin Trans. 2* **1995**, *1*, 103; (b) Murashima, T.; Tsujimoto, S.; Yamada, T.; Miyazawa, T.; Uno, H.; Ono, N.; Sugimoto, N. *Tetrahedron Lett.* **2005**, *46*, 113; (c) Gottumukkala, V.; Ongayi, O.; Baker, D. G.; Lomax, L. G.; Vicente, M. G. H. *Bioorg. Med. Chem.* **2006**, *14*, 1871.
14. (a) Laville, I.; Pigaglio, S.; Blais, J. C.; Doz, F.; Loock, B.; Maillard, P.; Grierson, D. S.; Blais, J. *J. Med. Chem.* **2006**, *49*, 2558; (b) Tomé, J. P. C.; Neves, M. G. P. M. S.; Tomé, A. C.; Cavaleiro, J. A. S.; Mendonça, A. F.; Pedago, I. N.; Duarte, R.; Valdeira, M. L. *Bioorg. Med. Chem.* **2005**, *13*, 3878; (c) Chen, X.; Drain, C. M. *Drug Desig. Rev.-Online* **2004**, *1*, 215; (d) Ahmed, S.; Davoust, E.; Savoie, H.; Boa, A. N.; Boyle, R. W. *Tetrahedron Lett.* **2004**, *45*, 6045; (e) Chen, X.; Hui, L.; Foster, D. A.; Drain, C. M. *Biochemistry* **2004**, *43*, 10918.
15. (a) Kieda, C.; Monsigny, M. Invas. Metast.. *6* **1986**, 347; (b) Monsigny, M.; Roche, A. C.; Midoux, P.; Kieda, C.; Mayer, R. In *Lectins and Glycoconjugates in Oncology: Structure, Function, Clinical Application*; Gabius, H. J., Nagel, G. A., Eds.; Springer: Heidelberg, 1988; Ballut, S.; Makky, A.; Michel, J. P.; Maillard, P.; Rosilio, V. *Chem. Commun.* **2009**, 224.
16. Hao, E.; Jensen, T. J.; Vicente, M. G. H. *J. Porphyrins Phthalocyanines* **2009**, *13*, 51.
17. Wang, C.; Delcros, J. G.; Biggerstaff, J.; Phanstiel, O. *J. Med. Chem.* **2003**, *46*, 2672.
18. (a) Cohen, M.; Cullis, P. M.; Hartley, J. A.; Mather, A.; Symons, M. C. R.; Wheelhouse, R. T. J. *Chem. Soc., Chem. Commun.* **1992**, 298; (b) Ghaneolhosseini, H.; Tjarks, W.; Sjöberg, S. *Tetrahedron* **1998**, *54*, 3877; (c) Papadopoulou, M. V. R.; Bloomer, H. S. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1519; (d) Wallace, M. H.; Niiranen, K. *Amino Acids* **2007**, *33*, 261; (e) Xie, S.; Cheng, P.; Liu, G.; Ma, Y.; Zhao, J.; Chehtane, M.; Khaled, A. R.; Phanstiel, O., IV; Wang, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4471; (f) Bolognesi, M. L.; Calonghi, N.; Mangano, C.; Masotti, L.; Melchiorre, C. *J. Med. Chem.* **2008**, *51*, 5463; (g) Wang, J.; Xie, S.; Li, Y.; Guo, Y.; Ma, Y.; Zhao, J.; Phanstiel, O., IV; Wang, C. *Bioorg. Med. Chem.* **2008**, *16*, 7005.
19. (a) Phanstiel, O., IV; Price, H. L.; Wang, L.; Juusola, J.; Kline, M.; Shah, S. M. *J. Org. Chem.* **2000**, *65*, 5590; (b) Vigayanathan, V.; Thomas, T.; Shiranta, A.; Thomas, T. J. *Biochemistry* **2001**, *40*, 13644; (c) Carlisle, D. L.; Devereux, W. L.; Hacker, A.; Woster, P. M.; Casero, R. A. *J. Clin. Cancer Res.* **2002**, *8*, 2684.
20. Garcia, G.; Sarrazy, V.; Sol, V.; Le Morvan, C.; Granet, R.; Alves, S.; Krausz, P. *Bioorg. Med. Chem.* **2009**, *17*, 767.
21. (a) Helberger, J. H. *Justus Liebigs Ann. Chem.* **1937**, *529*, 205; (b) Helberger, J. H.; von Rebay, A.; Hever, D. B. *Justus Liebigs Ann. Chem.* **1938**, *533*, 197; (c) Helberger, J. H.; Hever, D. B. *Justus Liebigs Ann. Chem.* **1938**, *536*, 173.
22. (a) Barret, P. A.; Linstead, R. P.; Rundall, F. G.; Tuey, G. A. P. *J. Chem. Soc.* **1940**, 1079; (b) Linstead, R. P.; Weiss, F. T. *J. Chem. Soc.* **1950**, 2975.
23. Remy, D. E. *Tetrahedron Lett.* **1983**, *24*, 1451.
24. Bonnett, R.; McManus, K. A. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2461.
25. (a) Nguyen, L. T.; Senge, M. O.; Smith, K. M. *J. Org. Chem.* **1996**, *61*, 998; (b) Lash, T. D. *J. Porphyrins Phthalocyanines* **1997**, *1*, 29.
26. (a) Vicente, M. G. H.; Jaquinod, L.; Khoury, R. G.; Madrona, A. Y.; Smith, K. M. *Tetrahedron Lett.* **1999**, *40*, 8763; (b) Vicente, M. G. H.; Tomé, A. C.; Walter, A.; Cavaleiro, J. A. S. *Tetrahedron Lett.* **1997**, *38*, 3639; (c) Lee, S. H.; Smith, K. H. *Tetrahedron Lett.* **2005**, *46*, 2009.
27. Ito, S.; Murashima, T.; Ono, N.; Uno, H. *Chem. Commun.* **1998**, 1661.
28. Morgan, A. R.; Pangka, V. S.; Dolphin, D. *J. Chem. Soc., Chem. Commun.* **1984**, 1047.
29. (a) Finikova, O. S.; Cheprakov, A. V.; Beletskaya, I. P.; Caroll, P. J.; Vinogradov, S. A. *J. Org. Chem.* **2004**, *69*, 522; (b) Filatov, M. A.; Lebedev, A. Y.; Vinogradov, S. A.; Cheprakov, A. V. *J. Org. Chem.* **2008**, *73*, 4175.
30. (a) Arnold, D. P.; Burgess-Dean, L.; Hubbard, J.; Rahman, M. A. *Aust. J. Chem.* **1994**, *47*, 969; (b) Haake, G.; Struve, D.; Montforts, F. P. *Tetrahedron Lett.* **1994**, *35*, 9703.
31. Lindsey, J. S.; Hsu, H. C.; Schreiman, IC. *Tetrahedron Lett.* **1986**, *27*, 4969.
32. Schuster, M.; Winter, K.; Hermann, Z. *Naturforsch* **1986**, *41*, 511.
33. Sol, V.; Blais, J. C.; Carré, V.; Granet, R.; Guilloton, M.; Spiro, M.; Krausz, P. *J. Org. Chem.* **1999**, *64*, 4431.
34. Schneider, R.; Schmitt, F.; Frochot, C.; Fort, Y.; Lourette, N.; Guillemin, F.; Müller, J. F.; Barberi-Heyob, M. *Bioorg. Med. Chem.* **2005**, *13*, 2799.
35. (a) Trombotto, S.; Bouchu, A.; Descotes, G.; Queneau, Y. *Tetrahedron Lett.* **2000**, *41*, 8273; (b) Listkowski, A.; Ing, P.; Cheaib, R.; Chambert, S.; Doutheau, A.;

Queneau, Y. *Tetrahedron: Asymmetry* **2007**, *18*, 2201; (c) Queneau, Y.; Chambert, S.; Besset, C.; Cheaib, R. *Carbohydr. Res.* **2008**, *343*, 1999.
36. Porter, C. W.; Bergeron, R. J.; Stolowich, N. J. *Cancer Res.* **1982**, *42*, 4072.
37. Sol, V.; Lamarche, F.; Enache, M.; Garcia, G.; Granet, R.; Guilloton, M.; Blais, J. C.; Krausz, P. *Bioorg. Med. Chem.* **2005**, *14*, 1364.
38. (a) Barber, D. C.; Freitag-Beeston, R. A.; Whitn, D. C. *J. Phys. Chem.* **1991**, *95*, 4074; (b) Furhop, J. H.; Demoulin, C.; Boettcher, C.; Koning, J.; Siggel, U. *J. Am. Chem. Soc.* **1992**, *114*, 4159.
39. Csik, G.; Balog, E.; Voska, I.; Tölgyesi, F.; Oulmi, D.; Maillard, P.; Momenteau, M. *J. Photochem. Photobiol., B* **1998**, *44*, 216.
40. Tomé, J. P. C.; Silva, E. M. P.; Pereira, A. M. V. M.; Alonso, C. M. A.; Faustino, M. A. F.; Neves, M. G. P. M. S.; Tomé, A. C.; Cavaleiro, J. A. S.; Tavares, S. A. P.; Duarte, R. R.; Caeiro, M. F.; Valdeira, M. L. *Bioorg. Med. Chem.* **2007**, *15*, 4705.
41. (a) Ohloff, G. *Pure Appl. Chem.* **1975**, *43*, 481; (b) Albro, P. W.; Corbett, J. T.; Shroeder, J. L. *Photochem. Photobiol.* **1994**, *60*, 310; (c) Böcking, T.; Barrow, K. D.; Netting, A. G.; Chilcott, T. C.; Coster, H. G.; Höfer, M. *Eur. J. Biochem.* **2000**, *267*, 1607.
42. (a) Indig, G. L.; Anderson, G. S.; Nichols, M. G.; Bartlett, J. A.; Mellon, W. S.; Sieber, F. *J. Pharm. Sci.* **2000**, *89*, 88; (b) Scalise, I.; Durantini, E. N. *J. Photochem. Photobiol., A* **2004**, *162*, 105.
43. Momenteau, M.; Maillard, P.; De Bélinay, M. A.; Carrez, D.; Croisy, A. *J. Biomed. Opt.* **1999**, *4*, 298.
44. Momenteau, M.; Oulmi, D.; Maillard, P.; Croisy, A. *Photodyn. Ther. Cancer II, Proc. SPIE* **1994**, *2325*, 13.
45. Banfi, S.; Caruso, E.; Caprioli, S.; Mazzagatti, L.; Canti, G.; Ravizza, R.; Gariboldi, M.; Monti, E. *Bioorg. Med. Chem.* **2004**, *12*, 4853.