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Synthesis and photophysical characterization of a library of photostable halogenated bacteriochlorins: an access to near infrared chemistry

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ABSTRACT

Near infrared (NIR) photons are ideally suited for photomedicine because they are relatively harmless and penetrate deeply in biological tissues. However, their use is impaired by lack of straightforward methods to synthesize large quantities of stable infrared-absorbing molecules with long-lived excited states. Here we present a one-step synthesis of amphiphilic *meso*-phenyl halogenated bacteriochlorins, via hydrazide reduction, possessing strong absorption about 750 nm. The reaction proceeds efficiently, in large quantities, with a solid–solid solvent-free methodology, that is characterized by its simplicity, efficiency and minimum environmental impact. The new bacteriochlorins have unprecedented chemical and photophysical properties, namely strong electronic absorption above 720 nm, adequate photostability, low fluorescence quantum yield and *n*-octanol/water partition coefficients (log P_{OW}) ranging from −1.7 to >4, meaning that the library of compounds synthesized in this work is versatile enough to be applied in photodynamic therapy for a range of biological targets.

1. Introduction

Near infrared (NIR) absorbing materials are of great importance for several photochemical applications. Over the past years this type of compounds has been used for several applications, such as optical recording, thermal writing displays, laser printers and filters, infrared photography, photovoltaic cells and photomedicine.[1] Our particular interest in photomedicine[2] led us to design molecules with strong electronic absorption bands in the infrared but nearly transparent in the visible part (380–720 nm) of the electromagnetic spectrum. Intense infrared absorption is desirable in imaging and photodynamic therapy (PDT) of cancer, because NIR photons in the phototherapeutic window (720–900 nm) are the most penetrating and least harmful to human tissues.[3]

Medicinal applications of NIR photons have been limited by the lack of chromophores possessing stability, solubility, synthetic flexibility and tuneable photophysical properties. Bacteriochlorophylls and bacteriopheophytins are examples of natural compounds with intense absorption bands above 720 nm, but their lability is well known.[4,5] Synthetic analogues, such as tetraphenylbacteriochlorins (TPB), retain the strong absorption in the NIR,[6,7] but their widespread use is also limited by their sensitivity towards oxidative photobleaching.[8–10]

The main focus of this work is to prepare improved photosensitizers for PDT of cancer. This form of therapy requires the accumulation of a photosensitizer in hyperproliferative cells, its excitation in the phototherapeutic window and efficient energy transfer to oxygen. Singlet oxygen ($^1O_2$) and other reactive oxygen species are formed and triggers cell death through a cascade of reactions in its local environment,[11,12] thus minimizing the side effects of the therapy. Porphyrin-like photosensitizers, such as Photofrin® (a purified mixture of hematoporphyrin derivatives)[13] and Foscan® (tetrahydroxyphenyl chlorin, THPC)[14] have enjoyed general acceptance in clinical PDT,[13–16] although their long wavelength absorption maxima and molar extinction coefficients are less than ideal. These photophysical limitations were overcome by Tookad® (a palladium/bacteriopheophorbide),[17] currently in clinical trials,[18] but at the expense of loss of stability. This work describes the synthesis of amphiphilic halogenated sulfonamide bacteriochlorins with intense longwavelength absorption bands, ca. 750 nm, photostabilities approaching those of chlorins and porphyrins and capable of efficiently photogenerating singlet oxygen.

Efforts to synthesize bacteriochlorins with the properties described above and capable of enduring standard laboratory procedures

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have been guided by three observations: (i) appropriate metals in the tetrapyrrolic ring increase its oxidation potential and stabilize the bacteriochlorins against oxidation, as in metallobacteriochlorophylls;[19,20] (ii) geminal dialkyl groups in each reduced pyrroline ring lock-in the reduction level of the bacteriochlorins, as in tolyporphin A;[21] (iii) exocyclic rings in chlorins impart stability towards oxidation,[22–25] Fig. 1. Our approach to make more stable bacteriochlorins started with the introduction of electron-withdrawing groups in the *meso*-tetraphenylbacteriochlorins, guided by the increase in the oxidation potential of zinc tetraphenylporphyrin (ZnTPP) derivatives by 0.17 or 0.27 eV when eight Cl or eight F atoms are present in the *ortho*-positions of its phenyl rings, respectively. This is a much higher increase than that measured, for example, in the metallation of H_2TPP to PdTTP, 0.07 eV[26–29]

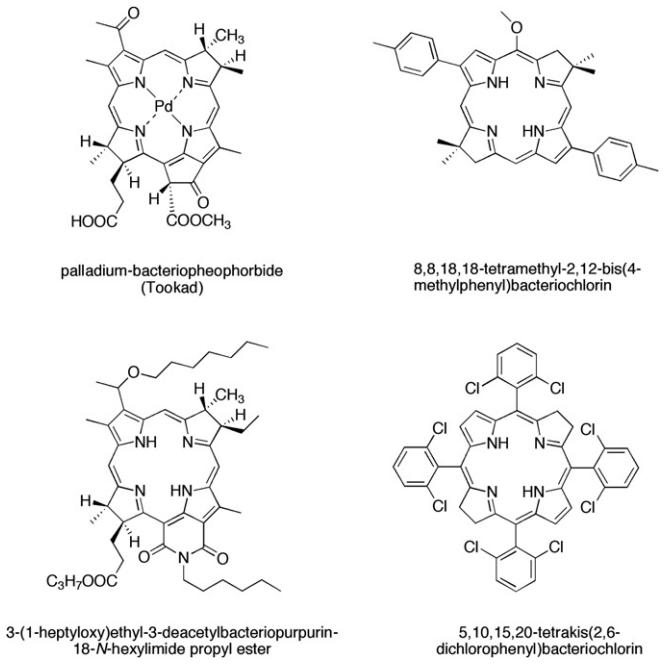


Fig. 1. Examples of bacteriochlorins stabilized by a metal, geminal dialkyl groups, a fused imide ring, or by electron-withdrawing substituents.

In addition to the thermodynamic stability expected from the increase of the oxidation potential, we also pursue the kinetic stability associated with steric protection of labile bonds. Bulky substituents and long or branched sulfonamides should provide steric hindrance against oxidation with confinement of the less reactive conformations.[30] Additionally, the choice of halogens as substituents introduces internal heavy-atom effects that promote intersystem crossing to the triplet state,[29] which is the precursor to the formation of cytotoxic species.[7] Derivatization with sulfonamides also responds to another determinant of PDT efficacy: controlled lipophilicity, as measured by *n*-octanol/water partition coefficients (log P_{OW}).[31]

The peripheral double bonds of the porphyrin macrocycle exhibit properties of normal alkenes due to their partial isolation from the macrocyclic conjugation pathway, and most of the approaches to synthesize bacterichlorins involve chemical derivatizations of the rings B and D of the corresponding porphyrins. There are four main routes to synthesize bacteriochlorins:[32–34] (i) derivatization of naturally occurring porphyrins, chlorins or bacteriochlorins; (ii) derivatization of β-substituted synthetic porphyrins; (iii) total synthesis and (iv) diimide reduction of 5,10,15,20-substituted synthetic porphyrins. The last approach (Whitlock method)[35] is widely used for the preparation of hydroporphyrins, namely Foscan®.[36,37] However, it has several inconveniences: (i) use of organic solvents, (ii) organic or inorganic bases to promote diimide generation, (iii) repeated addition of hydrazide to obtain acceptable yields of bacteriochlorins. Moreover, the crude product is contaminated with substantial amounts of the corresponding chlorin that are very laborious to separate, as described by Bonnett.[36]

We recently reported the synthesis of *meso*-chlorinated sulfamoyl phenyl bacteriochlorins using a modification of the classic Whitlock method, where toluene was used as solvent and a hindered organic base as a catalyst.[2,38] We now approach that synthesis from the point of view of sustainable chemistry, acknowledging that the removal of organic solvents from chemical synthesis is an increasingly important issue for health, environment, energy, technology and renewable resources.[39] Toxic organic solvents are tentatively replaced by ionic liquids,[40,41] liquid and supercritical CO_2,[42] or water.[43,44] Alternatively, some organic reactions are carried out under 'solvent-free' conditions.[39] In this work, we describe an environmentally friendly synthesis of halogenated sulfonamide and sulfonated bacteriochlorins in solid–solid solvent-free conditions,[45] that is, characterized by its simplicity, efficiency and minimum environmental impact and capable of delivering a library of multi-gram pure, stable and versatile amphiphilic bacteriochlorins.

2. Results and discussion

2.1. Synthesis

The widespread use of new materials with customized properties is often limited by their availability. Having in mind the large-scale production of stable and versatile bacteriochlorins, for applications in photomedicine, we designed a bacteriochlorin synthesis from halogenated 5,10,15,20-tetraphenylporphyrin precursors, generally believed to be easier to scale-up.

The synthetic route from the halogenated tetraphenylporphyrins (TPP) to obtain multi-gram synthesis of bacteriochlorins was designed to involve four very efficient and scalable steps: (i) one-pot synthesis of halogenated tetraphenylporphyrins; (ii) chlorosulfonation to the corresponding 3- or 5-chlorosulfophenylporphyrins; (iii) nucleophilic attack with water or amines to give amphiphilic sulfonamide halogenated porphyrins; (iv) diimide solvent-free reduction of these amphiphilic porphyrins to the analogous bacteriochlorins.

Halogenated TPPs were prepared by one-pot condensation of pyrrole with the desired *ortho*-halogenated benzaldehyde using acetic acid/nitrobenzene as solvent and oxidant.[46–48] Chlorosulfonation of the halogenated tetraphenylporphyrin precursors, followed by nucleophilic substitution with water or amines gave the desired amphiphilic sulfonamide halogenated porphyrins.[31] The derivatized porphyrins were reduced to the corresponding bacteriochlorins using solid *p*-toluenesulfonylhydrazide, both as reagent and as solvent, as hydrogen source and in the absence of solvents or bases.

In a standard solid–solid reaction, the two solids, porphyrin (gram scale) and an excess of *p*-toluenesulfonylhydrazide (1:30) were mixed together and heated up to 150 °C for 10 min in the absence of oxygen, under vacuum. After cooling to room temperature the reactor was open to the air. A UV–vis spectrum of the crude, prior to any purification, was recorded. The high purity of the bacteriochlorins synthesized by this new solventless synthetic approach is clearly documented by the UV–vis spectra of the crude, Fig. 2.

Starting from *ortho* mono or di-halogenated porphyrins (Cl or F) and several amphiphilic sulfamoyl side chains (OH, NHMethyl, NdiMethyl, NHethyl and NHheptyl), this methodology afforded the desired library of amphiphilic bacteriochlorins. Purification of the bacteriochlorins was performed using a short silica gel column, to remove the hydrazide impurities, at room temperature, without solvent degasification and even under these conditions, the degradation is negligible. One other important point that should be emphasized, regarding the purification of the obtained bacteriochlorins, is the difference in polarity of the compounds. For instance, sulfonamide substituted bacteriochlorins (Cl_2BEt, Cl_2BHep, ClBEt, FBMet

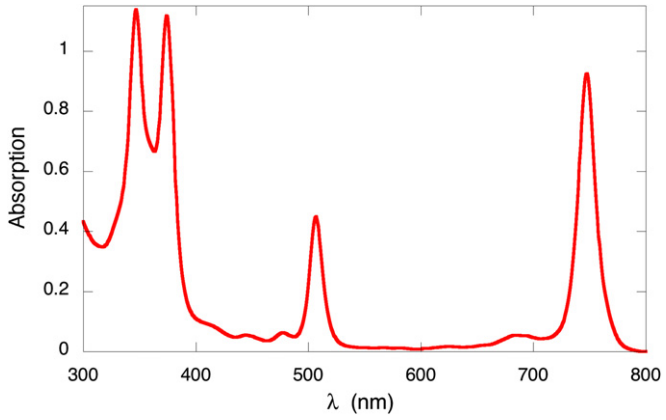


Fig. 2. UV–vis spectrum of the crude mixture in CH_2Cl_2 obtained from the synthesis of FBMet via diimide reduction in the absence of solvent and base. The band beginning at 300 nm is due to the excess *p*-toluenesulfonylhydrazide and its degradation products, and disappears with the work-up procedure.

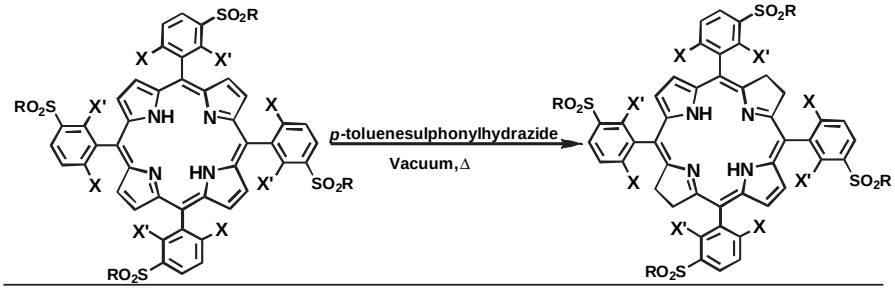
and $FBMet_2$) are much less polar than the sulfonated bacteriochlorins (ClBOH, Cl_2BOH and F_2BOH). In fact, while for the first case the crude mixture was purified by short column flash chromatography eluting with ethyl acetate/hexane (3:1), to remove the hydrazide derivatives, the more polar sulfonated bacteriochlorins were first washed with dichloromethane and then purified by flash chromatography eluting with a mixture of acetone/acetonitrile/methanol/triethylamine (14:6:1.5:1). The TPBs structures are illustrated in Fig. 3, together with the isolated reaction yields (ca. 80%). It should be highlighted that the isolated bacteriochlorins can be stored in the dark for several months without appreciable degradation.

The work-up required to remove the excess of hydrazide and its degradation products gives negligible oxidation of the bacteriochlorin under standard chromatography conditions. The isolated yields of the bacteriochlorins are neither dependent on the halogen atom in the *ortho*-positions of the phenyl ring, nor on the alkyl side chain of the sulfonamide group, Fig. 3. The excess of *p*-toluenesulfonylhydrazide present in our synthetic method compensates for the thermal decomposition of diimide,[54,55] ensuring that all the porphyrin is dissolved at 150 °C, under vacuum.

2.2. *n*-Octanol/water partition coefficients and photophysical properties

Partition coefficients in *n*-octanol/water (1:1, v:v), P_{OW}, are a convenient measure of polarity and solubility in biocompatible solvents. We measured P_{OW} using a minor modification of the shake-flask method,[56–58] recently described by us,[31] and present in Fig. 3 the corresponding logarithm values. The solutions for the calibration curves were either ethanol/*n*-octanol or ethanol/PBS, with precisely weighted amounts of the appropriate photosensitizer. The absorption band ca. 515 nm was excited and the fluorescence was collected in the NIR region, under the same instrumental conditions for both the calibration curve and the samples. The values of log P_{OW} of our bacteriochlorins are very similar to those of the corresponding porphyrins. The range of P_{OW} values covered is appropriate for biocompatible vehicles, meaning that the library of compounds synthesized in this work is versatile enough for PDT.

The absorption bands and the corresponding molar absorption coefficients of our TPB are presented in Table 1. The molar absorption coefficients are very high, as expected for bacteriochlorins. These samples may have different proportions of atropisomers (geometric isomers resulting from the different positions of the *meta* substituent relative to the bacteriochlorin plane), originated by the restricted rotation of the single bond at the *meso*-position of halogenated TPBs. For the analogous porphyrins it was shown that the atropisomer with all the sulfamoyl groups on the same side of the plane is the most polar atropisomer and has the



X	X'	R	Abbrev.	Isolated Yields (%)	log P_{OW}
Cl	Cl	OH	**1**-Cl_2BOH	77	-1.69
Cl	Cl	NHethyl	**2**-Cl_2BEt	85	1.83
Cl	Cl	NHheptyl	**3**-Cl_2BHep	85	>4
Cl	H	OH	**4**-ClBOH	70	-1.70
Cl	H	NHethyl	**5**-ClBEt	75	2.50
F	F	OH	**6**-F_2BOH	85	-1.44
F	H	NHmethyl	**7**-FBMet	83	2.66
F	H	Ndimethyl	**8**-$FBMet_2$	71	>4

Fig. 3. Synthesis, isolated yields, and logarithms of *n*-octanol/water partition coefficients of sulfonated and halogenated bacteriochlorins.

Despite the several methods described in the literature for the synthesis of bacteriochlorins from porphyrin precursors, which generally give the corresponding chlorins, as common by-products, with a rather troublesome separation,[34,49–52] in our case the correspondent chlorin contamination is almost negligible (<1% based on the ε values of related chlorins[53] at 420 nm), Fig. 2.

Table 1
Absorption and fluorescence properties of halogenated bacteriochlorins in ethanol, their singlet oxygen quantum yields and photobleaching quantum yields

	Absorption [a] λ(nm), ε (mM^{-1} cm^{-1})				Fluorescence λ (nm) Φ_F		$^1O_2$ Φ_Δ [b]	$\Phi_{pb}\times10^6$
	B_y	B_x	Q_x	Q_y				
1-Cl_2BOH	**351.5**	**376.5**	**515**	**744.5**	748	0.0062	0.85	—
	62	68	34	61				
2-Cl_2BEt	**351**	**377.5**	**514**	**745.5**	749	0.0081	0.66	6[c]
	100	96	55	97				
3-Cl_2BHep	**351**	**377.5**	**514.5**	**746**	749	0.0082	0.63	0.4[d]
	77	78	39	76				
4-ClBOH	**353.5**	**375.5**	**514**	**741.5**	745	0.0403	0.42	296[c]
	65	80	37	61				
5-ClBEt	**353**	**377**	**513**	**743**	746	0.0384	—	82[c]
	74	84	45	76				
6-F_2BOH	**352**	**377**	**515**	**745**	745	0.0233	0.44	—
	61	68	33	56				
7-FBMet	**350**	**375**	**510.5**	**742.5**	746	0.0589	0.63	81[c]
	60	66	28	62				

[a] Upper line (bold) is λ in nm, and lower line is ε in mM^{-1} cm^{-1}.
[b] Experimental accuracy: ±0.05.
[c] In PBS:methanol.
[d] In methanol.

highest value of ε in the red.[59] Considering that the purification step involved a short silica-gel column chromatography to remove the excess of hydrazide and its decomposition products, the samples used for the determination of the values of ε may not contain the total amount of the most polar atropisomer.

The internal heavy-atom effect assists halogenated porphyrins, chlorins and bacteriochlorins to generate triplet states with nearly unit quantum yields.[7,29,33,34] The low fluorescence quantum yields obtained for all the halogenated bacteriochlorins reported in this work are consistent with the expectation of large triplet quantum yields, Table 1.[60] The triplet state of the photosensitizer is the precursor of the formation of reactive oxygen species, namely singlet oxygen. Table 1 also presents the singlet oxygen quantum yields (Φ_Δ) measured in ethanol according to the procedure recently recommended by us that makes use of the linear dependence between singlet oxygen emission intensity and the energy of the exciting laser pulse at low laser energies.[60]

2.3. Photobleaching

In general, the combination of light and oxygen rapidly degrades bacteriochlorins in solution. Thus, the stability of bacteriochlorins and their photobleaching rates must be measured under these conditions, which are also those relevant for photomedicine. We measured the photobleaching rates of some of our bacteriochlorins under diode laser irradiation at 748 nm and verified that they are directly proportional to the laser intensity. Fig. 4 shows that such rates follow first-order kinetics. Photobleaching rates of photosensitizers are measured in different experimental conditions and are not convenient for direct comparison. For example, the photobleaching of tetrahydroxyphenyl porphyrin (THPP), chlorin (THPC, commercialized as Foscan®) and bacteriochlorin (THPB), collected from literature data in Table 2, were measured in methanol, under 780 mW argon laser irradiation at 514 nm[6] and that of Tookad®, obtained from literature data (Table 2), was measured in acetone under 778-nm diode laser irradiation (13 mW).[17] The standard measure of photostability is the photobleaching quantum yield, Φ_{pb}, defined as (initial rate of disappearance of photosensitizer molecules)/(initial rate of absorption of photons).[8,61]

The photostabilities of halogenated bacteriochlorins are one to three orders of magnitude higher than those of other bacteriochlorins. Remarkably, Cl_2BHep in methanol ($\Phi_{pb}=2\times10^{-7}$) is more

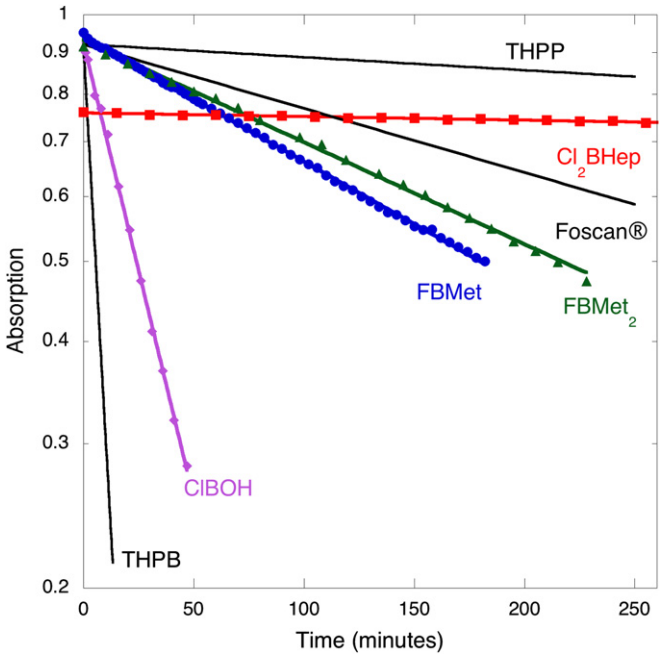


Fig. 4. Photostability of sulfonated and halogenated bacteriochlorins in aerated methanol solutions under 100 mW laser irradiation at 748 nm, and for the other photosensitizers under irradiation at 514 nm but normalized for the same number of absorbed photons as the bacteriochlorins.

Table 2
Photobleaching quantum yields calculated from literature data

	$\Phi_{pb}\times10^6$
THPP	3.8[a]
Foscan®	33[a]
THPB	1500[a]
Photofrin®	55[b]
Visudyne®	54[b]
Tookad®	1800[c]

[a] Calculated from data in Bonnett.[6]
[b] In PBS, according to Bonnett.[8]
[c] In acetone, calculated from data in Vakrat-Haglili.[17]

photostable than THPP ($\Phi_{pb}=3.8\times10^{-6}$), which is unprecedented for a bacteriochlorin and its Φ_{pb} is comparable to those of phthalocyanines.[8,61,62] It is generally believed that the ideal Φ_{pb} should be ca. 10^{-5}, which is within the range of those for the bacteriochlorins in the Table 1.[61]

PDT protocols often require light doses in excess of 100 J/cm^2, which in the standardized conditions of Fig. 4 correspond to 1000 s irradiation time. The photosensitizer should not bleach appreciably during the irradiation and this requirement suggests halogenated sulfonamide bacteriochlorins as the first choice for PDT photosensitizers. In fact, Fig. 4 shows that some of these bacteriochlorins are as photostable as the related porphyrins.

3. Conclusions

Our solvent-free synthesis of *meso*-halogenated phenyl bacteriochlorins can performed in a multi-gram scale via diimide reduction of the widely available *meso*-halogenated phenyl porphyrins in a single batch without difficulty.

The photobleaching quantum yields indicate that the halogens and sulfonyl electron-withdrawing groups in the phenyl ring positions of phenyl bacteriochlorins are an effective strategy to induce the desired stability in bacteriochlorins. Moreover, halogen atoms in the *ortho*-phenyl groups contribute to increase singlet oxygen quantum

yields via the internal heavy-atom effect in the intersystem crossing from the singlet to the triplet manifolds of the photosensitizer.

The *n*-octanol/water partition coefficients show that our library of bacteriochlorins includes photosensitizers that range from hydrophilic to lipophilic. This offers many opportunities for the routes and vehicles of administration, increasing their versatility towards a wide range of biological targets.

In summary, we have presented a one-step, solvent-free method for simple and scalable production of nearly transparent, photostable and amphiphilic sensitizers with strong NIR absorptions, that is, both economical and eco-friendly. The properties of these photosensitizers are appropriate for PDT of cancers.

4. Experimental

4.1. Instrumentation

^{1}H and ^{19}F NMR spectra were obtained using a Bruker Avance III 400 MHz spectrometer. The chemical shifts are given in parts per million (ppm) relative to tetramethylsilane at δ 0.00 ppm for proton spectra and relative to trifluoracetic acid at δ 0.00 ppm for ^{19}F spectra. Mass spectra (MALDI-TOF) were acquired using an Applied Biosystems Voyager DE-STR instrument equipped with a nitrogen laser (λ=337 nm). Elemental analysis was obtained with an EA1108-CHNS-0 Fisons Instruments. Flash column chromatography was performed with silica gel grade 60, 70–230 mesh.

4.2. Photophysical measurements

Absorption and fluorescence spectra were measured by standard techniques using Shimadzu UV-2100 and SPEX Fluoromax 3.22 spectrophotometers, respectively. The reference employed for fluorescence quantum yield measurements was 5,10,15,20-tetrakis (2,6-dichlorophenyl) bacteriochlorin, Φ_F=0.012 in toluene.[7]

Time-resolved singlet oxygen phosphorescence measurements were made with a modification of an Applied Photophysics LKS.60 flash photolysis spectrometer, and using the third harmonic of an Nd:YAG laser (Spectra-Physics Quanta Ray GCR 130, 5–6 ns FWHM) for excitation and HP Infinium (500 MHz, 1 GSa/s) or Tektronix DPO 7254 (2.5 GHz, 40 GSa/s) oscilloscopes. Singlet oxygen emission was detected using a Hamamatsu R5509-42 photomultiplier, cooled to 193 K in a liquid nitrogen chamber (Products for Research, model PC176TSCE005). Interposition of a Melles Griot cold mirror (03MCS005), that reflects more than 99% of the incident light in the 400–700 nm range and of a Scotch RG665 filter, eliminated from the infrared signal all harmonic contributions of the sensitizer emission in the 400–900 nm range. A 600 line diffraction grating was mounted in place of a standard one to improve spectral resolution and sensitivity in the NIR. This equipment allows for spectral identification of the singlet oxygen phosphorescence and measurement of singlet oxygen lifetime in the nanosecond and microsecond ranges. Extrapolating to time-zero the decays of the singlet molecular oxygen emissions (I_{Δ}^{0}) measured in ethanol for the reference (phenalenone, Φ_{Δ}=0.95±0.02) and for the bacteriochlorins, at a given laser intensity, we obtain a relation between emission intensities, that is identical to the relation between the singlet molecular oxygen quantum yields.[60] The actual singlet oxygen quantum yields were obtained from the linear dependence between I_{Δ}^{0} and the energy of the laser pulse $E_{h\nu}$, for the range of laser energies where a good linear relationship was found (correlation coefficient larger than 0.975). Good linearity was observed up to 6–7 mJ/pulse with fluorinated bacteriochlorins and up to 10–13 mJ/pulse for chlorinated bacteriochlorins.

4.3. *n*-Octanol/water partition coefficients

The *n*-octanol/water partition coefficients were measured following shake-flask method with minor modifications.[31,56–58] The sulfonamide halogenated bacteriochlorins (8×10^{-2} μmol) are dissolved in 5 mL of *n*-octanol previously saturated with a solution of PBS (pH 7.4). The same volume (5 mL) of a PBS solution, saturated with *n*-octanol, was added to the *n*-octanol phase, mixed vigorously for 3 min and then the phases were separated by centrifugation (4000 rpm, 2 min). Longer contact times between the phases were tested and shown not to influence the final results. One aliquot of 3 mL was taken from each phase and diluted with ethanol in order to attain the ratio ethanol/*n*-octanol (70:30) or ethanol/PBS (70:30). The fluorescence of each ethanol/*n*-octanol and ethanol/PBS solution was measured and compared with a calibration curve to obtain the concentration of the photosensitizer. The solutions for the calibration curves were either ethanol/*n*-octanol (70:30) or ethanol/PBS (70:30) and were both prepared in a range of concentrations between 0.1 μM and 1 μM. The absorption band ca. 515 nm was excited, and the fluorescence was collected in the NIR region, under the same instrumental conditions for both the solutions of the calibration curve and the samples. The determination of log P_{OW} for the sulfonic acid bacteriochlorin derivatives, F_2BOH and ClBOH, was carried out like the procedure described above, but initially the sensitizers have been dissolved in PBS solution previously saturated with *n*-octanol.

The partition coefficients were calculated from the ratio C_{oct}/C_{PBS}, where C_{oct} and C_{PBS} are the concentrations of bacteriochlorin in *n*-octanol and in PBS, respectively. All the measurements were carried out at room temperature.

4.4. Synthesis of the precursors

The synthesis of the porphyrin precursors followed the method of Pereira et al.[46–48] The chlorosulfonic derivatives were obtained by aromatic electrophilic chlorosulfonation followed by reaction with nucleophiles, such as water or amines.[31]

4.5. General procedure for solvent-free bacteriochlorin synthesis

The desired porphyrin (1 mmol) and *p*-toluenesulfonylhydrazide (30 mmol) were grinded into very fine powder and thoroughly mixed. The powder was introduced into a steel reactor equipped with a glass flask, which was evacuated to 0.1 Torr and kept for 2 h under these conditions. Next, the reactor was sealed and heated to 150 °C for 10 min, and then brought back to room temperature. The crude solid was dissolved in dichloromethane and contains a 99:1 ratio of bacteriochlorin/chlorin according to the UV–vis spectra. The crude was purified by flash chromatography eluting with ethyl lactate/hexane (1:4), to remove the hydrazide derivatives. The sulfonated bacteriochlorins were first washed with dichloromethane and then purified by flash chromatography eluting with a mixture of acetone/acetonitrile/methanol/triethylamine (14:6:1.5:1).

*4.5.1. 5,10,15,20-Tetrakis(2,6-dichloro-3-sulfophenyl) bacteriochlorin (**1**-Cl_2BOH).* Yield 0.934 g (77%); mp>300 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 8.42 (d, *J*=8.4 Hz, 4H), 7.98 (s, 4H), 7.88 (d, *J*=8.4 Hz, 4H), 4.05–3.93 (m, 8H), −1.26 (s, 2H); MS (MALDI-TOF): *m*/*z* 1213.0 $[M-H]^-$; elemental analysis calcd (%) for $C_{44}H_{26}Cl_8N_4O_{12}S_4$ $0.4^{+}NHEt_3\cdot6H_2O$: C 47.17, H 5.94, N 6.47, S 7.41, found: C 47.34, H 6.07, N 6.23, S 7.38.

*4.5.2. 5,10,15,20-Tetrakis(2,6-dichloro-3-N-ethylsulfamoylphenyl) bacteriochlorin (**2**-Cl_2BEt).* Yield 1.125 g (85%); mp 285–287 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.42 (d, *J*=8.4 Hz, 4H), 7,88 (d, *J*=8.4 Hz,

4H), 7.83–7.79 (m, 4H), 5.00 (m, 4H), 3.91 (s, 8H), 3.24–3.21 (m, 8H), 1.26–1.22 (m, 12H), −1.27 (s, 2H); HRMS (ESI): calcd for $C_{52}H_{47}Cl_8N_8O_8S_4$ $[M+H]^+$ 1322. 9855, found *m/z* 1322.9912; elemental analysis calcd (%) for $C_{52}H_{46}Cl_8N_8O_8S_4$: C 47.21, H 3.50, N 8.47, S 9.70; found: C 47.25, H 3.59, N 8.40, S 9.64.

*4.5.3. 5,10,15,20-Tetrakis(2,6-dichloro-3-N-heptylsulfamoylphenyl) bacteriochlorin (**3**-Cl$_2$BHep).* Yield 1.362 g (85%); mp 265–266 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (d, *J*=8.4 Hz, 4H), 7.88 (d, *J*=8.4 Hz, 4H), 7.83–7.82 (m, 4H), 5.00 (m, 4H), 3.90 (s, 8H), 3.15–3.10 (m, 8H), 1.30–1.26 (m, 40H), 0.86–0.83 (t, *J*=6 Hz, 12H), −1.27 (s, 2H); MS (MALDI-TOF): *m/z* 1602.3 $[M]^+$; elemental analysis calcd (%) for $C_{72}H_{86}Cl_8N_8O_8S_4$: C 53.93, H 5.41, N 6.99; found: C 54.17, H 5.62, N 7.23.

*4.5.4. 5,10,15,20-Tetrakis(2-chloro-5-sulfophenyl)bacteriochlorin (**4**-ClBOH).* Yield 0.754 g (70%); mp>300 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 8.38 (s, 4H), 8.21 (d, *J*=8.4 Hz, 4H), 8.04–8.02 (m, 4H), 7.98 (d, *J*=8.4 Hz, 4H), 4.16–4.02 (m, 8H), −1.26 (s, 2H); HRMS (ESI): calcd for $C_{44}H_{31}Cl_4N_4O_{12}S_4$ 1076. 9548 $[M+H]^+$, found *m/z* 1076.9543.

*4.5.5. 5,10,15,20-Tetrakis(2-chloro-5-N-ethylsulfamoylphenyl) bacteriochlorin (**5**-ClBEt).* Yield 0.887 g (75%); mp>300 °C; ^{1}H NMR (400 MHz, $CDCl_3$): 8.39–8.26 (m, 4H), 8.17–8.05 (m, 4H), 7.95–7.88 (m, 4H), 7.84–7.80 (m, 4H), 4.54–4.47 (m, 4H), 3.94 (m, 8H), 3.21–3.16 (m, 8H), 1.27–1.21 (m, 12H), −1.36 (s, 2H); HRMS (ESI): calcd for $C_{52}H_{51}Cl_4N_8O_8S_4$ 1183.1461 $[M+H^+]$, found *m/z* 1183.1325; elemental analysis calcd (%) for $C_{52}H_{50}Cl_4N_8O_8S_4$: C 52.70, H 4.25, N 9.46; found: C 52.21, H 4.55, N 9.42.

*4.5.6. 5,10,15,20-Tetrakis(2,6-difluoro-3-sulfophenyl) bacteriochlorin (**6**-F$_2$BOH).* Yield 0.921 g (85%); mp>300 °C; ^{1}H NMR (400 MHz, CD_3OD): δ 8.31–8.26 (m, 4H), 8.21 (s, 4H), 7.53–7.44 (m, 4H), 4.22–4.10 (m, 8H), −1.33 (s, 2H); ^{19}F NMR (376.5 MHz, CD_3OD): δ −108.2 to −108.3 (m, 4F), −108.5 to −108.6 (m, 4F); HRMS (ESI): calcd for $C_{44}H_{27}F_8N_4O_{12}S_4$ 1083.0375 $[M+H]^+$, found *m/z* 1083.0375.

*4.5.7. 5,10,15,20-Tetrakis(2-fluoro-5-N-methylsulfamoylphenyl) bacteriochlorin (**7**-FBMet).* Yield 0.882 g (83%); mp 290–291 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.28–8.24 (m, 4H), 8.13–8.11 (m, 4H), 7.88 (m, 4H), 7.53–7.48 (m, 4H), 4.45–4.36 (m, 4H), 3.97 (m, 8H), 2.76–2.75 (m, 12H), −1.45 (s, 2H); ^{19}F NMR (376.5 MHz, $CDCl_3$): δ −129.7 to −130.0 (m, 4F); MALDI-TOF (*m/z*): 1062.2 $[M]^+$; elemental analysis calcd (%) for $C_{48}H_{42}F_4N_8O_8S_4$: C 54.23, H 3.98, N 10.54; found: C 53.85, H 4.19, N 10.39.

*4.5.8. 5,10,15,20-Tetrakis(2-fluoro-5-N-dimethylsulfamoylphenyl) bacteriochlorin (**8**-FBMet$_2$).* Yield 0.795 g (71%); mp>300 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26 (dd, *J*=6.2 Hz, *J*=17.0 Hz, 4H), 8.12–8.10 (m, 4H), 7.96 (s, 4H), 7.61–7.59 (m, 4H), 4.05 (m, 8H), 2.91–2.79 (m, 24H), −1.40 (s, 2H); ^{19}F NMR (376.5 MHz, $CDCl_3$): δ −103.7 to −106.4 (m, 4F); HRMS(ESI): *m/z*: calcd for $C_{52}H_{51}F_4N_8O_8S_4$ 1119.2643 $[M+H]^+$; found 1119.2631.

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