JOC The Journal of Organic Chemistry

ARTICLE

pubs.acs.org/joc

Chlorin Photosensitizers Sterically Designed To Prevent Self-Aggregation

Adjaci F. Uchoa,[†,§,¶,*] Kleber T. de Oliveira,[‡] Mauricio S. Baptista,[§] Adailton J. Bortoluzzi,[⊥] Yassuko Iamamoto,[¶] and Osvaldo A. Serra[¶,*]

[¶]Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Avenida Bandeirantes 3900, 14040-901, Ribeirão Preto-SP, Brazil

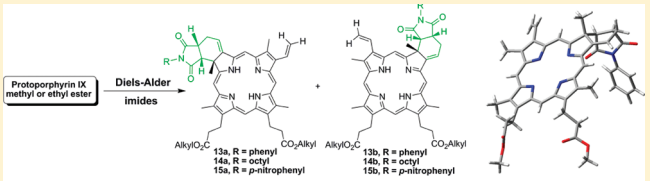
[§]Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Avenida Prof. Lineu Prestes 748, Cidade Universitária, 05508-000, São Paulo-SP, Brazil

[⊥]Departamento de Química, Centro de Ciências Físicas e Matemáticas, Universidade Federal de Santa Catarina Trindade 88040-900—Florianópolis-SC, Brazil

[‡]Departamento de Química, Universidade Federal de São Carlos, Rodovia Washington Luís, km 235 - SP-310, 13565-905, São Carlos-SP, Brazil

Supporting Information

ABSTRACT: The synthesis and photophysical evaluation of new chlorin derivatives are described. The Diels—Alder reaction between protoporphyrin IX dimethyl ester and substituted maleimides furnishes *endo*-adducts that completely prevent the self-aggregation of the chlorins. Fluorescence, resonant light scattering (RLS) and ^{1}H NMR experiments, as well as X-ray crystallographic have demonstrated that the configurational arrangement of the synthesized chlorins prevent π-stacking interactions between macrocycles, thus indicating that it is a nonaggregating photosensitizer with high singlet oxygen (Φ_Δ) and fluorescence (Φ_f) quantum yields. Our results show that this type of synthetic strategy may provide the lead to a new generation of PDT photosensitizers.



Endo chlorins self-prevented of aggregation

INTRODUCTION

Photodynamic therapy (PDT) is a consolidated technique for the treatment of cancer and other diseases.[1,2] It is based on the damage of living tissues by visible light in the presence of a photosensitizer and oxygen.[3−5] However, the action mechanism involved in PDT is still a matter of extensive study with respect to photosensitizer incorporation in biological systems,[6−9] to the relationship between the structure of the photosensitizer and the site of singlet oxygen generation,[10] and to the action mechanism of reactive oxygen species (ROS).[11] There are several photosensitizers in use today that are based on porphyrin, phenothiazinium, phthalocyanine, and chlorin derivatives. However, the future of PDT is highly dependent on the development of more efficient photosensitizers.[12] In addition to the photochemical yield, there are several factors that are important for PDT efficiency, such as tissue/cell localization and membrane binding.[13] Therefore, having a large photochemical yield is certainly a necessary, though not the sole, characteristic required to develop new PDT photosensitizers.[14]

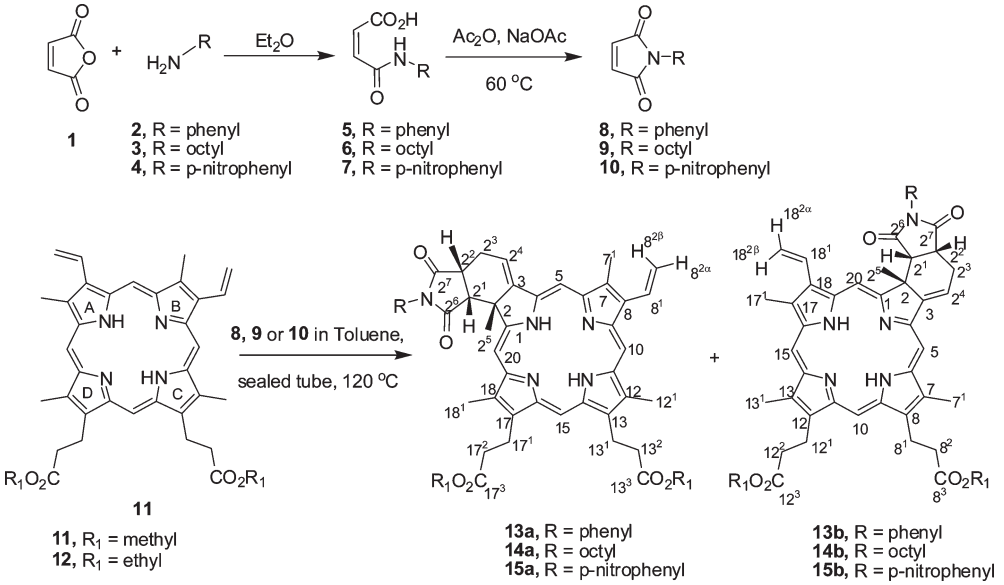
A factor that limits the efficiency of most photosensitizers is their tendency to self-aggregate via the strong attractive interactions between π-systems of the polyaromatic macrocycles. The geometries of π-conjugated systems induce strong van der Waals interactions, thus leading to self-aggregation in both the solution and solid state.[15−20] The interactions are affected mainly by the solvent, sample concentration, temperature, and specific interactions with biological structures.[15−26]

Administrated solutions used in PDT are developed to reduce photosensitizer aggregation by using emulsion/polymer preparations.[1−5] However, in the biological environment, it is difficult to control aggregation with these strategies. For example, interaction with membranes can concentrate photosensitizers, considerably increasing local concentration and consequently shifting the monomer/aggregate equilibrium to aggregation.[24−26] Therefore, the most relevant strategy would be to avoid aggregation in the target tissue, which can only be obtained by using molecules whose molecular structure avoids aggregation.[1−5,16−25]

In polar non-nucleophilic solvents such as chloroform, chlorophyll *a* exists mainly in the dimeric form, whereas larger aggregates are formed in nonpolar solvents like benzene or octane.[21−23] The same tendency has been observed for synthetic chlorins.[15−19] Such interactions are regarded as being due to $\pi-\pi$ and $\pi-\sigma$ interactions and are easily characterized using NMR spectroscopy.[27−31]

Received: July 28, 2011
Published: September 20, 2011

Scheme 1. Synthesis of New Chlorins (13−15) through a Diels-Alder Reaction



Self-aggregated states reduce fluorescence quantum yields, triplet states, and singlet oxygen generation, thereby diminishing photosensitizing activity.[32−34] In self-aggregated states, porphyrins adopt cofacial arrangements with their centers offset in both solution and solid state.[27−29] With the aim of reducing aggregation, several authors have proposed the use of bulky peripheric groups intended to reduce solute−solute and to enhance solute−solvent interactions.[21,35,36] The *tert*-butyl group has frequently been coupled to phthalocyanines and naphthalocyanines.[37] Picket fence porphyrins have also provided promising results.[38,39] To achieve this same effect, the menthyl group, which is reported to be bulkier and more efficient[35] than *tert*-butyl, has been used to synthesize new photosensitizers that are less prone to aggregate in solution. Interestingly some authors have shown strategies to actively manage aggregation by adding substituent groups that favor aggregation and disaggregation in the same molecules, that is, by adding groups that form hydrogen bonds and by avoiding planarity of the rings, respectively.[31,40−42] Additionally, the immobilization of photosensitizers on nanoparticle carriers has also been shown to avoid states of aggregation.[36,43−45]

All these strategies favor the presence of monomeric species of the photosensitizer in solution. However, no strategy has proven effective to the degree that only monomers are present even in concentrated solutions or in the presence of biological systems that favor aggregation. In general terms, all the aforementioned photosensitizer structures are somewhat planar, thus still allowing electronic cloud overlap and aggregation at higher concentrations.

From a synthetic viewpoint, the Diels−Alder reaction, a stereospecific reaction, has been efficiently employed to synthesize chlorin derivatives starting from protoporphyrin IX dimethyl ester as diene and dimethyl acetylene dicarboxylate or maleic anhydride as dienophiles.[46−50] The former dienophile led to the development of verteporfin, a potent photosensitizer and the most relevant commercial success in the area of PDT.[47] The latter dienophile is advantageous since it enables the insertion of different amphiphilic groups in the anhydride function.[50]

In this work, the Diels−Alder reaction between protoporphyrin IX dimethyl ester and substituted maleimides is described. Maleimide dienophiles[51] have been utilized in order to induce *endo* addition by secondary orbital stabilization. Compared to the other antiaggregation strategies we have proposed,[31] these new photosensitizers have a bulkier substituent and a smaller photosensitizer core, allowing the synthesis of new chlorin molecules that have a nonplanar and rigid "L"-type shape, which completely prevents aggregation, even at high concentrations (10^{-2} mol L^{-1}).

RESULTS AND DISCUSSION

Synthesis and Characterization. Maleimides **8**−**10** were synthesized according to the procedure described in the literature.[52] The Diels−Alder reactions between maleimides **8**−**10** and protoporphyrin IX dimethyl ester or protoporphyrin IX diethyl ester (**11** or **12**) were performed in a sealed tube by using different concentrations of maleimide solutions (3−20, equiv. to **8** or **9**), in dry/deoxygenated toluene at 120 °C (Scheme 1).

Chlorins **13**−**15** (Scheme 1) were obtained by using different concentrations of maleimides; however, the optimum concentrations of dienophiles were defined as 3 equiv of *N*-benzylmaleimide or *p*-nitrophenyl maleimide (**8** or **10**) and 6 equiv of *N*-octyl maleimide (**9**). The reactions were monitored by TLC and UV−vis at 666 nm, and the best results were achieved after 12 h for dienophile **8** and **10** and after 20 h for **9**. The products were purified using column chromatography (silica) followed by preparative TLC (silica), using a 25:1 mixture of $CHCl_3$/AcOEt as eluent, which furnished the pure isomers. Chlorins **13a** and **13b** have an R_f of 0.38 and 0.54, respectively; **14a** and **14b** have an R_f of 0.52 and 0.67, respectively; and **15a** and **15b** have an R_f of 0.28 and 0.42, respectively. An ^{1}H NMR spectra confirmed that each spot corresponded to a single compound. The amounts obtained and yields were as follows: **13a** and **13b**, 47 mg

dx.doi.org/10.1021/jo201568n |*J. Org. Chem.* 2011, 76, 8824–8832

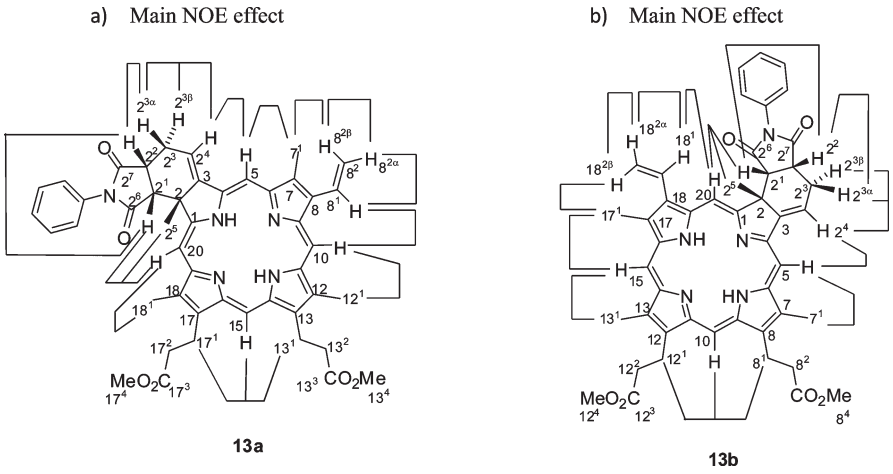


Figure 1. (a) Main NOE effects in compound **13a**; (b) main NOE effects in compound **13b**.

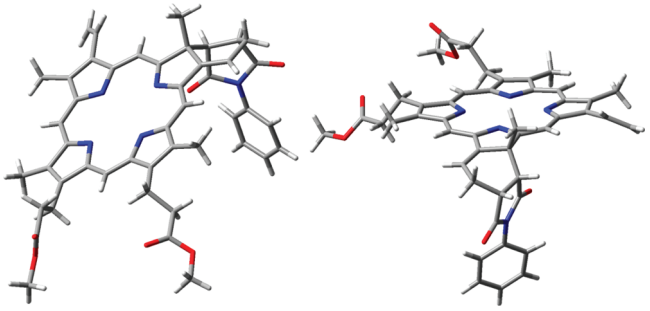


Figure 2. X-ray projections of compound **13b**.

(0.062 mmol) with a 72% yield; **14a** and **14b**, 40 mg (0.050 mmol) and a 58% yield; and **15a** and **15b**, 49 mg (0.058 mmol) with a 67% yield.

All compounds were fully characterized by 1D (^{1}H, ^{13}C and DEPT-135) and 2D (HMBC, HSQC, COSY, NOESY) NMR techniques, ESI-MS, UV−vis, resonant light scattering and photoluminescence. The regioselectivity of the Diels−Alder reaction (reaction in ring **A** or **B** of the porphyrin) and the stereochemistry (*cis-endo*) were deduced from the NOESY spectra. For example, Figure 1 details the main correlations for compounds **13a** and **13b**. Furthermore, the structure identified as **13b** by 1D and 2D NMR analysis was confirmed using X-ray crystallography (Figure 2).

Chlorins **13a** and **13b** presented an unequivocal signal in the ^{1}H NMR spectra at 4.65 ppm (doublet), corresponding to H-2^1 in both structures (Figure 1). Also, both products presented a nuclear overhauser effect (NOE) between H-2^1 and a *meso*-hydrogen referred to as H-20 in both compounds **13a** and **13b**. In the case of the product with a higher R_f, the *meso*-hydrogen H-20 presented an NOE with one vinylic hydrogen (double doublet at 8.12 ppm and J = 17.5 and 11.5 Hz), providing the first piece of evidence that this is isomer **13b** (Figure 1b). We concluded that the unique possibility of an NOE between a *meso*-hydrogen H-20 and a vinylic hydrogen (double doublet at 8.12 ppm and J = 17.5 and 11.5 Hz) occurs in compound **13b**, and the hydrogen with this chemical shift and these coupling constants has to be H-18^1 (Figure 1b). Other correlations observed by using NOE (Figure 1b) were also decisive in drawing conclusions about the regiochemistry and the stereochemistry of **13b**. For example, the NOE between H-2^1 (Figure 1b) and the distinguished methyl group H-2^5 (at 2.07 ppm) was decisive to assign the *cis-endo* structure of chlorin **13b**. The NOE between H-2^1 and H-2^2 was also used to confirm the *cis* structure of **13b** and was in agreement to the Diels−Alder reaction mechanism.

For the product with a lower R_f, that is, **13a**, we also observed a NOE between H-2^1 and H-20 and between H-2^1 and H-2^5; however, a NOE was also observed between the distinguished exocyclic vinylic hydrogen (triplet in 7.41 ppm and J = 4.9 Hz) H-2^4 and another *meso*-hydrogen, supposedly H-5. This supposed hydrogen H-5 presented a NOE with the methyl group H-7^1, and the hydrogen H-7^1 presented a NOE with the vinylic hydrogen H-$8^{2\beta}$ (doublet at 6.34 and J = 17.6 Hz), confirming the regiochemistry of this more polar isomer. The *cis-endo* structure of **13a** was also confirmed by a NOE between H-2^1 and H-2^5 and a NOE effect between H-2^1 and H2^2 (Figure 1a). Other NOE signals were useful to complete assignment of structure **13a**. The HMBC shows several C/H correlations ($^2J_{CH}$ and $^3J_{CH}$) that were decisive for the structural assignment of these isomers. There is correlation between C-2^6 with H-2^1 ($^2J_{CH}$) and 2^2 ($^3J_{CH}$). The C-2^7 shows correlation with H-2^2 ($^2J_{CH}$) and H-2^3 ($^3J_{CH}$). The hydrogens were assigned unambiguously by gCOSY. The HMBC spectra and the other 2D NMR analysis enabled a complete assignment of all protons and carbons for the two isomeric compounds. The complete assignments of spectroscopic data for chlorins are depicted in the Experimental Section. Therefore, we can conclude that the more polar compounds are **a**, and the less polar compounds are **b**. The spectra 2D NMR for **13a** and **13b** and all ^{1}H and ^{13}C NMR spectra are included in the Supporting Information.

UV−Visible and Fluorescence Emission. The UV−vis and fluorescence spectra were obtained in methanolic solutions. The new chlorins displayed typical UV−vis spectra with Soret and Q bands maximum absorption wavelength (λ_{max}) in nm (log ε) of 402 (5.1) and 666 (4.5), respectively. The correlation between Q/Soret bands was 0.22, with a Stokes shift of 5 nm. TPP, whose fluorescence quantum yield (Φ_f) in methanol is 0.11, was used as standard to quantify fluorescence yield. Φ_f values, which are shown in the inset of Figure 3, are related to the group inserted into the chlorin ring: R = phenyl, Φ_f = 0.16 for **13a**, and Φ_f = 0.17 for **13b**; R = octyl, Φ_f = 0.14 for **14a**, and Φ_f = 0.15 for **14b**; R = *p*-nitrophenyl, Φ_f = 0.12 for **15a**, and Φ_f = 0.13 for **15b**. All

dx.doi.org/10.1021/jo201568n |*J. Org. Chem.* 2011, 76, 8824–8832

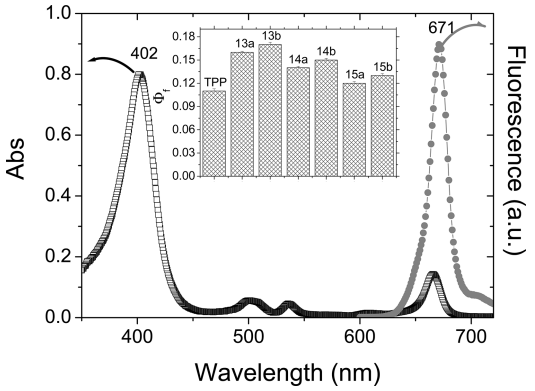


Figure 3. Absorption spectra in methanol (left) and fluorescence emission spectra in methanol (right) both of compound **13a**. Fluorescence quantum yield for the new chlorins. λ_{exc} = 500 nm; λ_{emi} = 510−720 nm (insert).

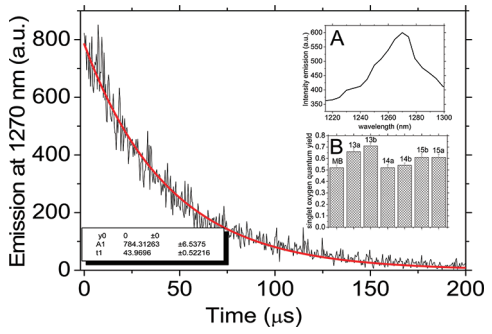


Figure 4. Transient emission at 1270 nm of compound **13a** in CH_3CN/H_2O (9:1) solution. Inserts: (A) emission spectrum of singlet oxygen of **13a**; (B) singlet oxygen quantum yield (Φ_Δ) in acetonitrile of compounds **13a**, **13b**, **14a**, **14b**, **15a**, **15b**, and methylene blue (MB). λ_{exc} = 532 nm; 10 Hz; 5 mJ/pulse.

compounds exhibit high Φ_f compared with related compounds, such as other chlorins and porphyrins, thus indicating stronger molecular rigidity.[53,54]

Singlet Oxygen Quantum Yield. Emission of singlet oxygen was characterized and quantified by the direct method (NIR phosphorescence emission) in a CH_3CN/H_2O 9:1 mixture, using an instrument and technique described earlier.[55] Figure 4 shows the transient at 1270 nm and spectra of singlet oxygen emission after exciting compound **13a** at 532 nm, as well as the singlet oxygen quantum yields of the new chlorins (insert). $^1O_2$ has a lifetime of 44 μs, which is shorter than its characteristic lifetime in pure acetonitrile (54.4 ± 1.3 μs),[56] but it is in agreement with the presence of water in the solvent mixture (CH_3CN/H_2O). The Φ_Δ values for the series were R = phenyl, Φ_Δ = 0.66 for **13a** and Φ_Δ = 0.71 for **13b**; R = octyl, Φ_Δ = 0.52 for **14a** and Φ_Δ = 0.54 for **14b**; R = *p*-nitrophenyl, Φ_Δ = 0.61 for **15a** and **15b**.

These results demonstrate that the new chlorins were able to generate singlet oxygen with high quantum yields. Chlorins with R = phenyl led to higher Φ_Δ because the structures of these chlorins (**13a** and **13b**) seem to be more rigid and to present fewer vibrational levels. Assuming that no other processes are involved, the yield of nonradiative decay (Φ_{nr}) can be estimated by adding Φ_Δ and Φ_t and subtracting the result from 1. Φ_{nr} was 0.18 and 0.12 for compounds **13a** and **13b**; 0.34 and 0.31 for compounds **14a** and **14b**; and 0.27 and 0.26 for **15a** and **15b**,

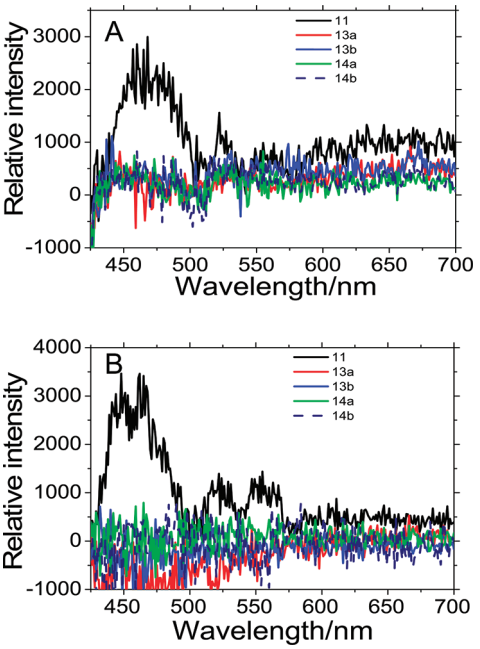


Figure 5. Resonant light scattering (RLS) spectra of the compounds **13a**, **13b**, **14a**, and **14b** and protoporphyrin IX dimethyl ester (**11**) (A) in $CHCl_3$ and (B) in $CH_3OH/CHCl_3$ 4:1, both at 10^{-4} mol L^{-1}, in a cuvette with 0.1 cm path length at room temperature.

respectively. The lower Φ_{nr} for compounds **13a** and **13b** is in agreement with the larger rigidity of the phenyl group compared to the other substituents.

Aggregation Studies of the New Chlorins. Self-aggregation was investigated by means of several methods and using protoporphyrin IX dimethyl ester as reference, which was chosen because it is the precursor of the new chlorins and because its solubility and spectroscopic properties are similar to those of the synthesized compounds.

Resonant light scattering (RLS) is a spectroscopic technique whose signal intensity depends on both excitonic coupling between light and the absorption cross section of the absorbing species and the size of these species, making it ideal to characterize aggregates of photosensitizer molecules.[22−24] The spectrum of protopophyrin IX dimethyl ester at 10^{-4} mol L^{-1} (**11**) displayed a very intense RLS peak near 460 nm, which corresponds to the λ_{max} of the J-band aggregate (Figure 5A,B).[15−19,21−23] None of the synthesized chlorins (**13a**, **13b**, **14a**, and **14b**) exhibited an RLS signal under the same experimental conditions, revealing that these compounds are free of self-aggregation at this concentration regime.[15−19,21−23]

In addition, to determine the possible formation of self-aggregates at high concentrations, the ^1H NMR technique was employed. It is known that the formation of aggregated states affects the diamagnetic anisotropy of porphyrin and chlorin rings.[21−23] The ^1H NMR spectra of protoporphyrin IX dimethyl ester at different concentrations (between 0.4 × 10^{-2} and 3.4 mol L^{-1}, Figure 6A) were analyzed with respect to changes in the chemical shifts as a function of concentration.[21−23,57] $\Delta\delta$-H shifts in the case of the *meso*-H of protoporphyrin IX dimethyl ester (Figure 6A, 10−8 ppm) demonstrated that aggregation is well pronounced in this case due to the formation of partially side-slipped face-to-face aggregates.[21−23,58]

dx.doi.org/10.1021/jo201568n |*J. Org. Chem.* 2011, 76, 8824–8832

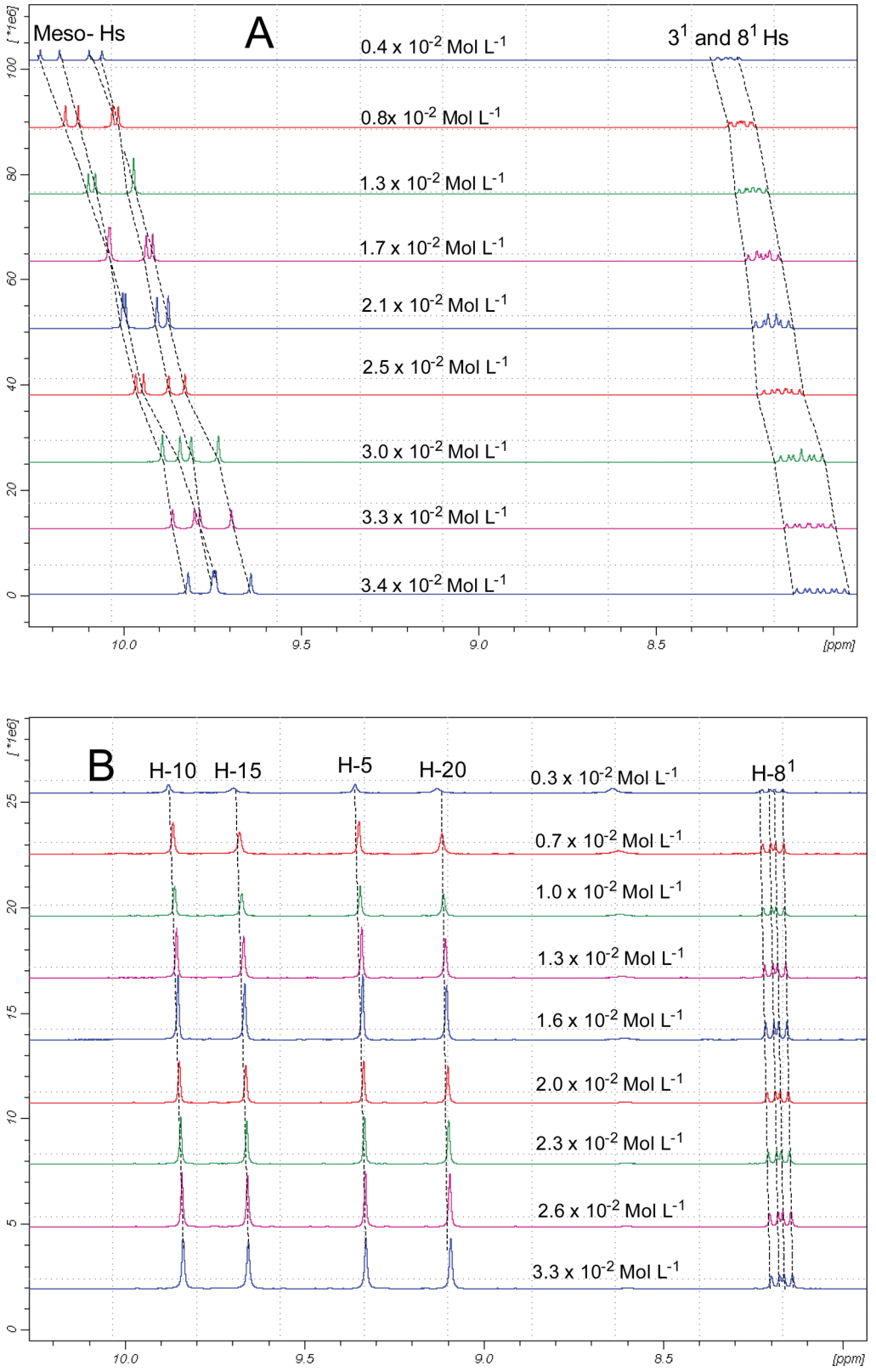


Figure 6. Changes in the chemical shifts of *meso* ^{1}H and vinylic hydrogen in $CDCl_3$ at 25 °C as a function of concentration of protoporphyrin IX dimethyl ester (A) and chlorin **13a** (B).

The spectra of the new apolar chlorins (**13**, **14**) were determined under the same experimental conditions. None of the spectra presented $\Delta\delta$-H shifts as a function of concentration. The data for chlorin **13a** are shown in Figure 6B and for compounds **13b**, **14a**, and **14b** are included in the Supporting Information, indicating that the new chlorins do

dx.doi.org/10.1021/jo201568n |*J. Org. Chem.* 2011, 76, 8824–8832

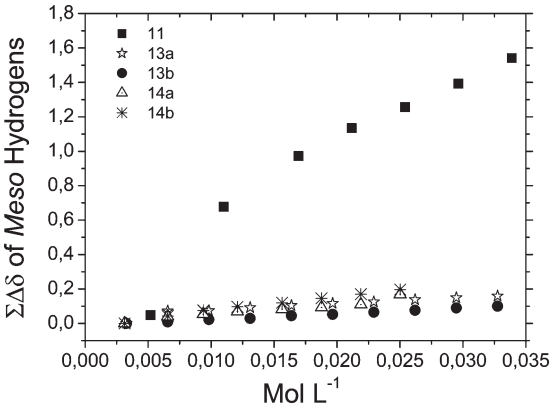


Figure 7. Sum of the chemical shifts of meso-hydrogens ($\Sigma\Delta\delta$) for compounds **11**, **13a**, **13b**, **14a**, and **14b** as a function of the compound concentration.

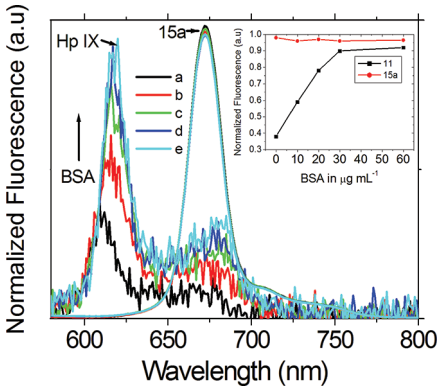


Figure 8. Fluorescence spectra of **Hp IX** (left) and **15a** (right) as a function of BSA concentration in μg mL^{-1} (a = 0, b = 10, c = 20, d = 30, e = 60) in a water solution. Insert: Fluorescence integral versus BSA concentration in μg mL^{-1}.

not undergo self-aggregation even in large concentration solutions.

To best view the changes in $\Delta\delta$-*meso*-H as a function of photosensitizer concentration, the sum of the chemical shifts of *meso*-hydrogen ($\Sigma\Delta\delta$-*meso*-H) as a function of concentration was calculated and plotted in Figure 7. Note that $\Sigma\Delta\delta$-*meso*-H is directly proportional to the concentration of protopophyrin IX dimethyl ester. In the case of the new compounds, there was no observable shift. It is important to emphasize that chlorins usually have a high tendency to form aggregates, often hindering the acquisition of the ^{1}H NMR spectra in $CDCl_3$.[35,50] The ^{1}H NMR data prove that the axial groups in chlorins **13a**, **13b**, **14a**, and **14b** definitely avoid self-aggregation.

Obtaining sensitizers with low levels of aggregation was our main goal.[8,35,36] Indeed, chlorins **13a**, **13b**, **14a**, and **14b** are completely free of aggregation, even at high concentrations. These results can be considered a major breakthrough in the development of photosensitizers, since the formation of aggregated states deactivates the excited state of the molecules, reducing the levels of singlet oxygen generation. The new molecular features were attained by employing strategic dienophiles, which induced the *endo* products and provided an "L"-type molecular scaffold that does not allow aggregation. However, these compounds have low aqueous solubility, and it is important to prove that this same concept could be applied in water solutions.

To test this hypothesis, two new "L"-type chlorins (**15a**, **15b**) having nitro substituents and increased aqueous solubility were prepared. Experiments were performed in the presence of a protein (BSA) because proteins are prone to induce photosensitizer aggregation or disaggregation depending on the photosensitizer/protein concentration ratio.[59] We used hematoporphyrin IX (**HP IX**) as reference because it has sufficient aqueous solubility.

The fluorescence spectra of both compounds were determined in the presence of increasing amounts of BSA. Note that, when BSA concentration is increased from 0 to 60 μg mL^{-1}, there is a large increase (~2.5 times) in the fluorescence emission in the case of Hp IX and almost no change in the case of **15a** (Figure 8 insert). The increase in fluorescence intensity indicates that BSA is able to dissociate the **Hp IX** aggregates that are present in pure water solutions, and the fact that there was no change in the emission profile of **15a** indicates that this molecule does not tend to aggregate in aqueous solution. Shifts in the maxima wavelengths are in agreement with this explanation. There were changes in the maxima of the emission (Figure 8) and absorption spectra (from 377 to 400 nm, data not shown) in the case of **Hp IX** with the increase of BSA concentration, which can also be explained by the disaggregation of HP IX, and there was no measurable change in the λ_{max} of emission and absorption of **15a**, showing that the concept of stabilization of monomeric species by the "L"-type scaffold is also valid in aqueous solution.

There are several works published in the literature showing axial substituent groups, and that bulky groups disfavor aggregation,[31,35,40−42] although no-one has obtained results of lack of aggregation in the high concentration regimes shown in this work. We think that the conformation of molecules **13**−**15** have such a high efficiency to prevent the interaction between π-systems, because of the extreme rigidity conferred by four sp^3 carbons in the same ring, at positions 2, 2^1, 2^2, and 2^3.

CONCLUSIONS

The new chlorins (**13**−**15**) have a bulky axial organic group and a relatively small porphyrin core, forming an "L"-type shape that hinders aggregation. These characteristics indicate that these compounds are potentially useful for PDT applications, as evidenced by the high singlet oxygen quantum yields and stability of the monomeric species in aqueous and organic media. The synthesis of these chlorins with substituted imides by Diels−Alder reactions can be considered an excellent and versatile method for the preparation of new chlorins that do not self-aggregate.

EXPERIMENTAL SECTION

General. Anhydrous toluene was purified according to a standard procedure.[50] Maleic anhydride was recrystallized in toluene, and other commercially available reagents were used without further purification. An ultrasound was employed for deoxygenation of the toluene. ^{1}H NMR and ^{13}C RMN spectra were recorded at 500.13 and 125.77 MHz, respectively, using $CDCl_3$ as the solvent and TMS as the internal reference. Unequivocal ^{1}H assignments were carried out by using 2D gCOSY (^{1}H/^{1}H) and NOESY spectra (mixing time of 800 ms). HRMS were obtained using the ESI technique. The UV−vis spectra were registered using methanol as the solvent. Flash chromatography was carried out by using silica gel 70−230 mesh, and the preparative thin layer chromatography was conducted on 20 × 20 cm glass plates coated

with silica gel/gypsum 60 (1 mm thick). Analytical TLC was performed on sheets precoated with silica gel (0.2 mm thick).

Syntheses of *N*-Phenylmaleimide (8), *N*-Octylmaleimide (9), and *P*-Nitrophenylmaleimide (10). To a solution of 0.196 g (0.02 mol) of maleic anhydride in ethyl ether (4 mL) at 0 °C, 0.02 mol of the corresponding amine (**2**, **3**, or **4**) was added, and the reactional mixture was stirred for 10 min. The white solid obtained was filtered off and washed with ethyl ether (2 × 10 mL). Then, intermediate **5**, **6**, or **7** was reacted in the presence of freshly distilled acetic anhydride (10 mL) and anhydrous sodium acetate (0.1 g) at 60 °C for 2 h. The reaction was poured into a water/ice mixture (100 mL) and then extracted with dichloromethane (2 × 100 mL). Compounds **8**, **9**, and **10** were purified by column chromatography on silica, using CH_2Cl_2/MeOH 9:1 as eluent.

***N*-Phenylmaleimide (8).** Compound (**8**) was obtained in a global 74% yield and was characterized by ^{1}H and ^{13}C NMR analysis: ^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 6.84 (br s, 2H, H-2 and H-3 vinyl group); 7.33−7.38 (m, H-7, H-8 and H-9); 7.45−7.48 (m, H-6 and H-10). ^{13}C NMR ($CDCl_3$, 125.77 MHz), δ (ppm): 126.0 (2C, C-6 and C-10); 127.9 (C-8); 129.1 (2C, C-7 and C-9); 131.2 (C-5); 134.2 (2C, C-2 and C-3); and 169.5 (2C, C-1 and C-4). ESI-MS-TOF, *m*/*z* 174.0550 calculated for $C_{10}H_8NO_2^+$ (MH^+); found, 174.0582.

***N*-Octylmaleimide (9).** Compound (**9**) was obtained in a global 52% yield and was characterized by ^{1}H and ^{13}C analysis: ^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 0.87 (t, 3H, *J* = 6.5 Hz, CH_3-12); 1.25−1.28 (m, 10H, H-7, H-8, H-9, H-10 and H-11); 1.56−1.58 (m, 2H, H-6); 3.50 (t, *J* = 7.0 Hz, 2H, H-5); 6.68 (br s, 2H, H-2 and H-3 vinyl group). ^{13}C NMR ($CDCl_3$, 125.77 MHz) δ (ppm): 14.0 (C-12); 22.6 (C-11); 26.7 (C-10); 28.5 (C-9); 29.0 (C-8); 29.1 (C-7); 31.7 (C-6); 37.9 (C-5); 134.0 (C-2 and C-3); 170.9 (C-1 and C-4). ESI-MS-TOF, *m*/*z* 210.1489 calculated for $C_{12}H_{20}NO_2^+$ (MH^+); found, 210.1495.

***P*-Nitrophenylmaleimide (10).** Compound (**10**) was obtained in a global 72% yield and was characterized by ^{1}H and ^{13}C NMR analysis: ^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): 6.93 (br s, 2H, H-2 and H-3 vinyl group); 7.69 (d, 2H, *J* = 10.0 H-6 and H-10); 8.34 (d, *J* = 10, H-7 and H-9). ^{13}C NMR ($CDCl_3$, 125.77 MHz), δ (ppm): 124.5 (2C, C-7 and C-9); 125.5 (2C, C-6 and C-10); 134.6 (2C, C-2 and C-3); 137.1 (C-5); 146.5 (C-8). ESI-MS-TOF, *m*/*z* 219.0400 calculated for $C_{10}H_7NO_4^+$ (MH^+); found, 219.0400.

General Procedure for the Diels−Alder Reaction. To a solution of maleimides **8**, **9**, or **10** (3 equiv for **8** and **10** or 6 equiv for **9**) in dry/deoxygenated toluene (5 mL), protoporphyrin IX diester (**11** or **12**) (42.3 mmol) was added. The reaction was performed in a sealed tube at 120 °C for 12 h in the case of dienophile **8** or **10**, and 20 h for dienophile **9**. After that, the reaction mixtures were directly poured into a column chromatograph (silica gel 70−230 mesh) and purified by using a 25:1 $CHCl_3$/AcOEt mixture as eluent. This preliminary purification was sufficient to isolate the isomeric mixture containing **13a**/**13b**, **14a**/**14b**, and **15a**/**15b**. The isomeric separations were carried out by preparative thin layer chromatography on 20 × 20 cm glass plates coated with silica gel 60 and gypsum (1 mm thick), yielding 72% of **13a**/**13b** isomers in a 1:1 ratio, 58% of **14a**/**14b**, 66% of **15a**/**15b** and isomers in the same proportion for all reactions.

2^1,2^2[*N*,*N*-Dicarbonyl-*N*-phenyl]-13,17-bis[2-(methoxycarbonyl)ethyl]-2,7,12,18-tetramethyl-8-vinyl-2,2^1,2^2,2^3-tetrahydrobenzo[*b*]porphyrin (13a). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ (ppm): −2.47 and −2.38 (br s, 2H, H-22 and H-24); 2.07 (s, 3H, CH_3-2^5); 3.15 (t, 2H, *J* = 7.8 Hz, H-13^2); 3.20 (t, 2H, *J* = 7.8 Hz, H-17^2); 3.40 (s, 3H, CH_3-12^1); 3.45−3.48 (m, 2H, H-$2^{3\alpha}$ and $2^{3\beta}$); 3.49 (s, 3H, CH_3-18^1); 3.56 (s, 3H, CH_3-7^1); 3.64 and 3.68 (2s, 3H and 3H; OCH_3-13^4 and OCH_3-17^4); 3.89−3.96 (m, 1H, H-2^2); 4.17 (t, 2H, *J* = 7.8 Hz, H-13^1); 4.29 and 4.30 (2dt,1H, J=7.8 and 4.4 Hz, H-$17^{1\alpha}$ and $17^{1\beta}$), 4.65 (d, 1H, *J* = 8.6 Hz, H-2^1); 6.14 (d, 1H, *J* = 11.5 Hz, H-$8^{2\alpha}$); 6.34 (d, 1H, *J* = 17.6 Hz, H-$8^{2\beta}$); 6.66−6.70 (m, 2H, H-2^9 and 2^{13}); 6.94−6.97 (m, 3H, H-2^{10},H-2^{11} and H-2^{12}); 7.41 (t, 1H, *J* = 4.9 Hz, H-2^4); 8.18 (dd, 1H, *J* = 17.6 and 11.5 Hz, H-8^1); 9.10 (s, 1H, H-20); 9.33 (s, 1H, H-5); 9.67 (s, 1H, H-15), 9.85 (s, 1H, H-10). ^{13}C NMR ($CDCl_3$, 125.77 MHz) δ (ppm): 11.4 (C-12^1); 11.6 (C-18^1); 12.3 (C-7^1); 21.5 (C-17^1); 21.9 (C-13^1); 25.6 (C-2^3); 26.5 (C-2^5); 36.6 (C-17^2); 37.0 (C-22); 38.5 (C-13^2); 50.1 (C-2^1); 51.6 (C-13^4); 51.8 (C-17^4); 52.3 (C-2); 90.5 (C-5); 93.4 (C-20); 97.9 (C-15); 99.8 (C-10); 115.6 (C-2^4); 121.3 (C-8^2); 126.0 (C-2^{10} and C-2^{12}); 128.1 (C-2^{11}); 128.6 (C-2^9 and C-2^{13}); 129.2 (C-7); 129.8 (C-8^1); 131.0 (C-18); 131.3 (C-2^8); 132.6 (C-9); 133.8 (C-8); 133.9 (C-16); 136.3 (C-17); 136.5 (C-12); 138.3 (C-6); 138.4 (C-19); 139.6 (C-13); 149.6 (C-14); 152.3 (C-4); 166.1 (C-1); 173.4 (C-13^3); 173.8 (C-17^3); 174.9 (C-2^7); 178.6 (C-2^6). ESI-MS-TOF, *m*/*z* 764.3443 calculated for $C_{46}H_{46}N_5O_6^+$ (MH^+); found, 764.3415.

2^1,2^2[*N*,*N*-Dicarbonyl-*N*-phenyl]-8,12-bis[2-(methoxycarbonyl)ethyl]-2,7,13,17-tetramethyl-18-vinyl-2,2^1,2^2,2^3-tetrahydrobenzo[*b*]porphyrin (13b). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ (ppm): −2.43 (br s, 2H, H-21 and H-23); 2.07 (s, 3H, CH_3-2^5), 3.16 (t, 2H, *J* = 7.7 Hz, H-12^2), 3.20 (t, 2H, *J* = 7.7 Hz, H-8^2); 3.41 (s, 3H, CH_3-13^1); 3.45−3.49 (m, 2H, H-$2^{3\alpha}$ and H-$2^{3\beta}$); 3.47 (s, 3H, CH_3-7^1); 3.60 (s, 3H, CH_3-17^1), 3.64 and 3.65 (2s, 3H and 3H; OCH_3-12^4 and OCH_3-8^4); 3.91−394 (m, 1H, H-2^2) 4.18 (t, 2H, *J* = 7.7 Hz, H-12^1); 4.32 (t, 2H, *J* = 7.7 Hz, H-8^1), 4.65 (d, 1H, *J* = 8.6 Hz, H-2^1); 6.10 (d, 1H, *J* = 11.5 Hz, H-$18^{2\alpha}$); 6.32 (d, 1H, J=17.5 Hz, H-$18^{2\beta}$) 6.65−6.72 (m, 2H, H-2^9 and 2^{13}); 6.92−6.99 (m, 3H, H-2^{10}, H-2^{11} and H-2^{12}); 7.42 (t, 1H, *J* = 5.0 Hz, H-2^4) 8.12 (dd, 1H, *J* = 17.5 and 11.5 Hz, H-18^1); 9.27 and 9.28 (s, 2H, H-5 and, H-20); 9.70 (s, 1H, H-10), 9.75 (s, 1H, H-15). ^{13}C NMR ($CDCl_3$, 125.77 MHz) d (ppm): 11.2 (C-13^1); 11.7 (C-7^1); 12.4 (C-17^1); 21.6 (C-8^1); 21.9 (C-12^1); 25.6 (C-2^3); 26.5 (C-2^5); 36.7 (C-8^2); 37.0 (C-12^2); 38.6 (C-2^2); 50.1 (C-2^1); 51.6 (C-12^4); 51.7 (8^4); 52.2 (C-2); 90.0 (C-5); 94.2 (C-20); 98.4 (C-10); 99.4 (C-15); 116.0 (C-2^4); 120.9 (C-18^2); 126.0 (C-2^9 and 2^{13}); 128.1 (C-2^{11}); 128.5 (C-2^{10} and 2^{12}); 129.9 (C-18^1); 131.3 (C-9); 131.4 (C-17); 133.4 (C-16); 133.9 (C-8); 136.1 (C-19); 137.5 (C-13); 137.9 (C-7); 139.1 (C-12); 139.7 (C-14); 149.7 (C-11); 151.2 (C-4); 163.9 (C-6); 165.9 (C-1); 173.4 (C-8^3); 173.8 (C-12^3); 174.7 (C-2^6); 178.6 (C-2^7). ESI-MS-TOF, *m*/*z* 764.3443 calculated for $C_{46}H_{46}N_5O_6^+$ (MH^+); found, 764.3422.

2^1,2^2[*N*,*N*-Dicarbonyl-*N*-octhyl]-13,17-bis[2-(methoxycarbonyl)ethyl]-2,7,12,18-tetramethyl-8-vinyl-2,2^1,2^2,2^3-tetrahydrobenzo[*b*]porphyrin (14a). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ (ppm): −2.46 and −2.39 (br s, 2H, H-22 and H-24); 0.55−0.67 (m, 9H, H-2^{15}, 2^{14}, H-2^{13} and H-2^{12}); 0.71−0.77 (m, 2H, H-2^{11}); 0.82−0.90 (m, 2H, H-2^{10}); 0.93−1.01 (m, 2H, H-2^9); 2.03 (s, 3H, CH_3-2^5); 3.00 (m, 2H, H-2^8); 3.16 (t, 2H, *J* = 7.6 Hz, H-13^2); 3.22 (t, 2H, *J* = 8.0 Hz, H-17^2); 3.35−3.39 (m, 2H, H-$2^{3\alpha}$ and H-$2^{3\beta}$); 3.40 (s, 3H, CH_3-7^1); 3.51 (s, 3H, CH_3-18^1); 3.53 (s, 3H, CH_3-12^1); 3.66 and 3.70 (2s, 3H and 3H; OCH_3-13^4 and OCH3−17^4); 3.73−3.76 (m, 1H, H-2^2); 4.18 (t, 2H, *J* = 8.0 Hz, H-131); 4.32 (t, 2H, *J* = 7.3 Hz, H-171); 4.46 (d, 1H, *J* = 8.4 Hz, H-2^1); 6.14 (d, 1H, *J* = 11.5 Hz, H-$8^{2\alpha}$); 6.34 (d, 1H, *J* = 17.7 Hz, H-$8^{2\beta}$); 7.34 (t, 1H, *J* = 5.0 Hz, H-2^4); 8.18 (dd, 1H, *J* = 17.7 and 11.5 Hz, H-8^1); 9.08 (s, 1H, H-20); 9.28 (s, 1H, H-5); 9.68 (s, 1H, H-15), 9.84 (s, 1H, H-10). ^{13}C NMR ($CDCl_3$, 125.77 MHz) d (ppm): 11.4 (C-12^1); 11.6 (C-18^1); 12.2 (C-7^1); 13.8 (C-2^{15}); 21.5 (C-17^2); 21.9 (C-13^2); 22.2 (C-2^{14}); 25.5 (C-2^3); 26.1 (C-2^{10}); 26.5 (C-2^5); 27.1 (C-2^9); 28.6 (2C, C-2^{12} and C-2^{11}); 31.4 (C-2^{13}); 36.6 (C-17^2); 37.1 (C-2^8); 38.3 (C-22); 38.7 (C-13^2); 49.9 (C-2^1); 51.6 (C-13^4); 51.8 (C-17^4); 52.2 (C-2); 90.3 (C-5); 93.4 (C-20); 97.9 (C-15); 99.7 (C-10); 115.6 (C-2^4); 121.2 (C-8^2); 129.1 (C-7); 129.8 (C-8^1); 130.9 (C-18); 132.5 (C-9); 133.7 (C-8); 133.9 (C-16); 136.3 (C-17); 136.5 (C-9); 138.3 (C-6); 139.6 (C-19); 149.4 (C-14); 151.0 (C-16); 151.3 (C-9); 152.3 (C-4); 166.3 (C-1); 173.4 (C-13^3); 173.8 (C-17^3); 175.8 (C-2^7); 179.6 (C-2^6). ESI-MS-TOF, *m*/*z* 800.4382 calculated for $C_{48}H_{58}N_5O_6^+$ (MH^+); found, 800.4389.

dx.doi.org/10.1021/jo201568n |*J. Org. Chem.* 2011, 76, 8824–8832

2^1,2^2[*N*,*N*-Dicarbonyl-*N*-octhyl]-8,12-bis[2-(methoxycarbonyl)ethyl]-2,7,13,17-tetramethyl-18-vinyl-2,2^1,2^2,2^3-tetrahydrobenzo[*b*]porphyrin (14b). ^{1}H NMR ($CDCl_3$, 500.13 MHz) δ (ppm): −2.43 (br s, 2H, H-21 and H-23), 0.58−0.64 (m, 9H, H-2^{15}, 2^{14}, H-2^{13} and H-2^{12}) 0.76−0.89 (m, 4H, H-2^{10} and H-2^{11}); 0.94−0.99 (m, 2H, H-2^9); 2.01 (s, 3H, CH_3-2^5), 2.98 (t, 2H, J = 7.5 Hz, H-2^8); 3.16 (t, 2H, J = 7.7 Hz, H-12^2), 3.17 (t, 2H, J = 7.7 Hz, H-8^2); 3.34−3.40 (m, 2H, H-2^3); 3.39 (s, 3H, CH_3-13^1); 3.43 (s, 3H CH_3-7^1); 3.59 (s, 3H, CH_3-17^1); 3.65 and 3.66 (2s, 3H and 3H; OCH_3-12^4 and OCH_3-8^4); 3.69−3.74 (m, 1H, H-2^2); 4.16 (t, 2H, J = 7.5 Hz, H-12^1); 4.29 (t, 2H, J = 7.5 Hz, H-8^1); 4.43 (d, 1H, J = 8.0 Hz, H-2^1); 6.11 (d, 1H, J = 11.2 Hz, H-$18^{2\alpha}$); 6.34 (d, 1H, J = 18.0 Hz, H-$18^{2\beta}$); 7.33 (t, 1H, J = 5.6 Hz, H-2^4), 8.14 (dd, 1H, J = 18.0 and 11.2 Hz, H-18^1); 9.20 (s, 1H, H-5); 9.25 (s, 1H, H-20); 9.66 (s, 1H-10), 9.72 (s, 1H, H-15). ^{13}C NMR ($CDCl_3$, 125.77 MHz) δ (ppm): 11.2 (C-13^1); 11.6 (C-7^1); 12.4 (C-17^1); 13.8 (C-2^{15}); 14.1 (CH_3); 21.5 (C-8^1); 21.9 (C-12^1); 22.2 (C-2^{14}); 26.1 (C-2^3); 26.5 (C-2^5); 27.0 (C-2^9), 28,6 (2 signals C2^{12} and C-2^{11}); 29.7 (C-2^{10}); 31.4 (C-2^{13}); 36.7 (C-8^2); 37.1 (C-2^8); 38.3 (C-2^2); 38.7 (C-8^2); 49.9 (C-2^1); 51.6 (C-12^4); 51.7 (8^4); 52.1 (C-2); 89.8 (C-5); 94.1 (C-20); 98.3 (C-10); 99.3 (C-15); 115.9 (C-2^4); 120.7 (C-18^2); 130.0 (C-18^1); 131.1 (C-9); 133.3 (C-17); 133.4 (C-16); 133.8 (C-8); 135.9 (C-19); 136.8 (C-12); 137.3 (C-13); 137.9 (C-7); 139.7 (C-14); 149.5 (C-11); 150.5 (C-3); 151.4 (C-4); 153.0 (C-6); 165.9 (C-1); 173.4 (C-8^3); 173.8 (C-12^3); 175.6 (C-2^6); 179.6 (C-2^7). ESI-MS-TOF, m/z 800.4382 calculated for $C_{48}H_{58}N_5O_6^+$ (MH^+); found, 800.4377.

2^1,2^2[*N*,*N*-Dicarbonyl-*N*-(4-nitrophenyl)]-13,17-bis[2-(ethoxycarbonyl)ethyl]-2,7,12,18-tetramethyl-8-vinyl-2,2^1,2^2,2^3-tetrahydrobenzo[*b*]porphyrin (15a). ^{1}H NMR ($CDCl_3$, 500 MHz),δ (ppm) −2.45 and −2.36 (br s, 2H, H-22 and H-24);1.17 and 1.18 (2t, 6H, CH_3-13^5 and CH_3-17^5); 2.11 (s, 3H, CH_3-2^5); 3.14 (t, 2H, J = 7.5 Hz, H-13^2); 3.19 (t, 2H, J = 7.5 Hz, H-17^2); 3.40 (s, 3H, CH_3-12^1); 3.44−3.49 (m, 2H, H-$2^{3\alpha}$ and $2^{3\beta}$); 3.50 (s, 3H, CH_3-18^1); 3.55 (s, 3H, CH_3-7^1); 3.97−4.01 (m, 1H, H-2^2); 4.12−4.18(m, 6H, H-13^1, H-13^4 e H-17^4); 4.32 (m, 2H,J=7.0 Hz H-17^1) 4.71 (d, 1H, J = 8.5 Hz, H-2^1); 6.16 (dd, 1H, J = 11.5 and 1.5 Hz, H-$8^{2\alpha}$); 6.35 (dd, 1H, J = 17.7 and 1.5 Hz, H-$8^{2\beta}$); 6.99 (d, J = 9.5 2H, H-2^9 and 2^{13}); 7.42 (t, 1H, J = 3.5 Hz, H-2^4); 7.82 (d, J = 9.5 2H, H-2^{10} and H-2^{12}); 8.18 (dd, 1H, J = 17.7 and 11.5 Hz, H-8^1); 9.08 (s, 1H, H-20); 9.33 (s, 1H, H-5); 9.71 (s, 1H, H-15), 9.87 (s, 1H, H-10). ^{13}C NMR ($CDCl_3$, 125.77 MHz) δ (ppm): 11.4 (C-12^1); 11.7 (C-18^1); 12.3 (C-7^1); 14.1 (C-17^5); 14.2 (C-13^5), 19.9 (C-17^1), 21.5 (C-13^1) 21.8 (C-17^1); 22.7 (C-13^1); 25.7 (C-2^3); 26.4 (C-2^5); 36.8 (C-17^2); 37.2 (C-2^2); 38.6 (C-13^2); 50.2 (C-2^1); 52.3 (C-2); 60.4 (C-17^4); 60.6 (C-13^4); 90.4 (C-5); 93.1 (C-20); 98.2 (C-15); 99.9 (C-10); 115.3 (C-2^4); 121.4 (C-8^2); 123.7 (C-2^{10} and C-2^{12}); 126.5 (C-2^9 and C-2^{13}); 129.4 (C-7); 129.7 (C-8^1); 130.0 (C-18); 130.9 (C-2^8); 132.6 (C-9); 133.8 (C-8); 134.0 (C-16); 136.4 (C-17); 136.6 (C-12);136.8 (C-11);138.3 (C-6); 138.5 (C-19); 139.9 (C-13); 146.5 (C-2^{11}); 149.7 (C-14); 151.5 (C-4); 151.8 (C-3); 165.4 (C-1); 172.9 (C-13^3); 173.3 (C-17^3); 174.1 (C-2^7); 177.9 (C-2^6). **ESI-MS-TOF**, m/z 837.3606 calculated for $C_{48}H_{49}N_6O_8^+$ (MH^+); found, 837.3616.

2^1,2^2[*N*,*N*-Dicarbonyl-*N*-(4-nitrophenyl)]-8,12-bis[2-(methoxycarbonyl)ethyl]-2,7,13,17-tetramethyl-18-vinyl-2,2^1,2^2,2^3-tetrahydrobenzo[*b*]porphyrin (15b). ^{1}H NMR ($CDCl_3$, 500 MHz), δ (ppm): −2.45 (br s, 2H, H-21 and H-23); 1.15 and 1.17 (2t, 6H, CH_3-12^5 and CH_3-8^5); 2.10 (s, 3H, CH_3-2^5), 3.15 (t, 2H, J = 8.0 Hz, H-12^2), 3.19 (t, 2H, J = 8.0 Hz, H-8^2); 3.43 (s, 3H, CH_3-13^1); 3.47 (s, 3H, CH_3-7^1); 3.48−3.50 (m, 2H, H-$2^{3\alpha}$ and H-$2^{3\beta}$);3.62 (s, 3H, CH_3-17^1), 3.95−4.00 (m, 1H, H-2^2) 4.10−4.19(m, 6H, H-12^1, H-12^4 e H-8^4); 4.32 (t, 2H, J = 8.0 Hz, H-8^1), 4.68 (d, 1H, J = 8.5 Hz, H-2^1); 6.12 (dd, 1H, J = 11.5 and 1.5 Hz, H-$18^{2\alpha}$); 6.34 (dd, 1H, J = 17.5 and 1.5 Hz, H-$18^{2\beta}$); 6.97 (d, J = 9.0 2H, H-2^9 and 2^{13}); 7.41−7.43 (m, 1H, H-2^4); 7.80 (d, 2H, J = 9.0, H-2^{10} and H-2^{12}); 8.11 (dd, 1H, J = 17.5 and 11.5 Hz, H-18^1); 9.27 (s, 2H, H-5 and, H-20); 9.74 (s, 1H, H-10), 9.78 (s, 1H, H-15). ^{13}C NMR ($CDCl_3$, 125.77 MHz) δ (ppm): 11.3 (C-13^1); 11.7 (C-7^1); 12.4 (C-17^1); 14.1 (C-17^5); 14.2 (C-13^5); 21.5 (C-8^1); 21.8 (C-12^1); 25.6 (C-2^3); 26.6 (C-2^5); 36.8 (C-8^2); 37.2 (C-12^2); 38.7 (C-2^2); 50.1 (C-2^1); 52.2 (C-2); 60.6 (C-12^4); 60.6 (8^4); 90.1 (C-5); 94.4 (C-20); 98.7 (C-10); 99.6 (C-15); 115.8 (C-2^4); 120.7 (C-18^2); 129.8 (C-2^8); 129.9 (C-18^1); 123.4 (C-2^{10} and C-2^{12}); 126.5 (C-2^9 and C-2^{13}); 133.6 (C-16); 133.9 (C-8); 136.8 (C-19); 146.5 (C-2^{11});149.8 (C-4); 172.9 (C-8^3); 173.3 (C-12^3); 174.0 (C-2^6); 177.8 (C-2^7). ESI-MS-TOF, m/z 837.3606 calculated for $C_{48}H_{49}N_6O_8^+$ (MH^+); found, 837.3623.

Spectroscopic and Photophysical Studies. UV−vis, fluorescence, and RLS spectra were obtained in conventional spectrophotometer and spectrofluorometer equipments. Extinction coefficients were obtained by measuring absorption spectra from 350 to 700 nm and plotting them as a function of the chlorin concentration. Fluorescence spectra were obtained in a methanol solution, excited at 500 nm, and detected from 530 to 800 nm. Fluorescence quantum yields (Φ_f) were determined by measuring the area under the emission spectra (530−800 nm range), using TPP in methanol (Φ_f = 0.11) as standard. RLS were recorded using the spectrofluorometer operating in synchronous scan mode, that is by simultaneously scanning the excitation and emission monochromators ($\Delta\lambda$ = 0 nm) of the spectrofluorometer from 350 to 700 nm in a cell with a path length of 0.1 cm. In all cases, the absorbance values of the sample and reference solutions were kept below 0.1 at the excitation wavelength to minimize inner filter effects. For studies of self-aggregation in water, Hp IX and **15a** were dissolved in DMSO. A 300 μL portion of this solution was added to a cuvette with a 1.0 cm path length containing 3 mL of water. The absorption and fluorescence spectra were obtained before and after addition of increasing amounts of the BSA solution of 3 mg mL^{-1}, 10, 20, 30, and 60 μL.

The quantum yield of singlet oxygen production (Φ_Δ) was calculated by using a phosphorescence detection method. A Nd:YAG laser operating at 532 nm (5 ns, 10 Hz) was used as the excitation source. The radiation emitted at 1270 nm was detected at a right angle by a liquid-nitrogen cooled NIR photomultiplier.[55] Three different concentrations of chlorin in CH_3CN/H_2O 9:1 were tested. The absorbance of the sample and standard (methylene blue)[53] were kept the same. Φ_Δ values were calculated by comparing the emissions of samples and standards.

■ ASSOCIATED CONTENT

Supporting Information. ^{1}H NMR and ^{13}C NMR spectra of compounds **8**, **9**, **10**, **13a**, **13b**, **14a**, **14b**; **15a**, and **15b**. The X-ray crystallographic (CIF) file is available. Figures of ^{1}H NMR studies about aggregation for compounds **13b**, **14a**, and **14b** have also been included. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: adjaci@usp.br; osaserra@usp.br.

Present Addresses

[†]Centro de Engenharia Biomédica, Universidade Camilo Castelo Branco, Parque Tecnológico de São José dos Campos, Rodovia Presidente Dutra, Km 138, 12247-004, São José dos Campos-SP, Brazil.

■ ACKNOWLEDGMENT

The authors wish to thank the FAPESP, CNPq, and CAPES for financial support of this research.

REFERENCES

(1) Bonnett, R.; Martinez, G. *Tetrahedron* **2001**, *57*, 9513.
(2) Tardivo, J. P.; Giglio, A. D.; Oliveira, C. S.; Gabrielli, D. S.; Junqueira, H. C.; Dayane, B. T.; Severino, D.; Turchiello, R. F.; Baptista, M. S. *Photodiagn. Photodyn. Ther.* **2005**, *2*, 175.
(3) Dolmans, D. E. J. G. J.; Fukumura, D.; K., J. R. *Nat. Rev. Cancer* **2003**, *3*, 380.
(4) Ochsner, M. *J Photochem. Photobiol. B* **1997**, *39*, 1.
(5) Dougherty, T. J. *Adv. Photochem.* **1992**, *17*, 275.
(6) Pavani, C.; Uchoa, A. F.; Oliveira, C. S.; Iamamoto, Y.; Baptista, M. S. *Photochem. Photobiol. Sci.* **2009**, *8*, 233.
(7) Celli, J. P.; Spring, B. Q.; Rizvi, I.; Evans, C. L.; Samkoe, K. S.; Verma, S.; Pogue, B. W.; Hasan, T. *Chem. Rev.* **2010**, 2795–2838. *Chem. Rev.* **2010**, *110*, 2795.
(8) Uchoa, A. F.; Oliveira, C. S.; Baptista, M. S. *J. Porphyrins Phthalocyanines* **2010**, *14*, 832.
(9) Chowhary, R. K.; Sharif, I.; Chansarkar, N.; Dolphin, D.; Ratakay, L.; Delany, S.; Meadows, H. *J. Pharm. Pharm. Sci.* **2003**, *6*, 198.
(10) Kuimova, M. K.; Yahioglu, G.; Ogilby, P. R. *J. Am. Chem. Soc.* **2009**, *131*, 332.
(11) Riske, K. A.; Sudbrack, T. P.; Archilha, N. L.; Uchoa, A. F.; Schroder, A. P.; Marques, C. M.; Baptista, M. S.; Itri, R. *Biophys. J.* **2009**, *97*, 1362.
(12) Savellano, M. D.; Hasan, T. *Photochem. Photobiol.* **2003**, *77*, 431.
(13) Engelmann, F. M.; Mayer, I.; Gabrielli, D. S.; Toma, H. E.; Kowaltowski, A. J.; Araki, K.; Baptista, M. S. *J. Bioenerg. Biomembr.* **2007**, *39*, 175.
(14) Castano, A. P.; Demidova, T. N.; Hamblin, M. R. *Photodiagn. Photodyn. Ther.* **2005**, *2*, 1.
(15) Helmich, F.; Lee, C. C.; Nieuwenhuizen, M. M. L.; Gielen, J. C.; Christianen, P. C. M.; Larsen, A.; Fytas, G.; Leclere, P. E. L. G.; Schenning, A. P. H. J.; Meijer, E. W. *Angew. Chem., Int Ed.* **2010**, *49*, 3939.
(16) Escudero, C.; C., J.; Díez-Pérez, I.; El-Hachemi, Z.; Ribó, J. M. *Angew. Chem., Int. Ed.* **2006**, *45*, 8032.
(17) Aggarwal, L. P. F.; Borissevitch, I. E. *Spectrochim. Acta, Part A* **2006**, *63*, 227.
(18) Huber, V.; Katterle, M.; Lysetska, M.; Wurthner, F. *Angew. Chem., Int. Ed.* **2005**, *44*, 3147.
(19) Aveline, B. M.; Hasan, T.; Redmond, R. W. *J. Photochem. Photobiol. B* **1995**, *30*, 161.
(20) Kasha, M.; Rawls, H. R.; El-Bayoumi, M.-A. *Pure Appl. Chem.* **1965**, *11*, 371.
(21) Kano, K.; K., F.; Wakami, H.; Nishiyabu, R.; Pasternack, R. F. *J. Am. Chem. Soc.* **2000**, *122*, 7494.
(22) Fong, F. K.; Koester, V. J. *J. Am. Chem. Soc.* **1975**, *97*, 6888.
(23) Gandini, S. C. M.; Gelamo, E. L.; Itri, R.; Tabak, M. *Biophys. J.* **2003**, *85*, 1259.
(24) Severino, D.; Junqueira, H. C.; Gugliotti, M.; Gabrielli, D. S.; Baptista, M. S. *Photochem. Photobiol.* **2003**, *77*, 459.
(25) Gabrielli, D.; Belisle, E.; Severino, D.; Kowaltowski, A. J.; Baptista, M. S. *Photochem. Photobiol.* **2004**, *79*, 227.
(26) Junqueira, H. C.; Severino, D.; Dias, L. G.; Gugliotti, M. S.; Baptista, M. S. *Phys. Chem. Chem. Phys.* **2002**, *4*, 2320.
(27) Hunter, C. A.; Leighton, P.; Sanders, J. K. M. *J. Chem. Soc., Perkin Trans. 1* **1989**, 547.
(28) Hunter, C. A.; Meah, M. N.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5773.
(29) Anderson, H. L.; Hunter, C. A.; Meah, M. N.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5780.
(30) Abraham, R. J.; Byrne, J. J.; Griffiths, L.; Perez, M. *Magn. Reson. Chem.* **2006**, *44*, 491.
(31) Abraham, R. J.; Smith, K. M. *J. Am. Chem. Soc.* **1983**, *105*, 5734.
(32) Hunter, C. A.; Sanders, J. K. M. *J. Am. Chem. Soc.* **1990**, *112*, 5525.
(33) Zenkevicha, E.; Saguna, E.; Knyukshtoa, V.; Shulgaa, A.; Mironov, A.; Efremovab, O.; Bonnett, R.; Songcac, S. P.; Kassemd, M. *J. Photochem. Photobiol. B* **1996**, *33*, 171.
(34) Ricchelli, F. *J. Photochem. Photobiol. B* **1995**, *29*, 109.
(35) de Oliveira, K. T.; de Assis, F. F.; Ribeiro, A. O.; Neri, C. R.; Fernandes, A. U.; Baptista, M. S.; Lopes, N. P.; Serra, O. A.; Iamamoto, Y. *J. Org. Chem.* **2009**, *74*, 7962.
(36) Rossi, L. M.; Silva, P. R.; Vono, L. L.; Fernandes, A. U.; Tada, D. B.; Baptista, M. S. *Langmuir* **2008**, *24*, 12534.
(37) Sibrian-Vazquez, M.; Ortiz, J.; Nesterova, I. V.; Fernandez-Lazaro, F.; Sastre-Santos, A.; Soper, S. A.; Vicente, M. G. *Bioconjugate Chem.* **2007**, *18*, 410.
(38) Barber, D. C.; VanDerMeid, K. R.; Gibson, S. L.; Hilf, R.; Whitten, D. G. *Cancer Res.* **1991**, *51*, 1836.
(39) Lawrence, D. S.; Gibson, S. L.; Nguyen, M. L.; Whittemore, K. R.; Whitten, D. G.; Hilf, R. *Photochem. Photobiol.* **1995**, *61*, 90.
(40) Barkigia, K. M.; Renner, M. W.; Senge, M. O.; Fajer, J. *J. Phys. Chem. B* **2004**, *108*, 2173.
(41) Shi, X. X.; Barkigia, K. M.; Fajer, J.; Drain, C. M. *J. Org. Chem.* **2001**, *66*, 6513.
(42) Nurco, D. J.; Smith, K. M.; Fajer, J. *Chem. Commun. (Cambridge)* **2002**, 2982.
(43) Prasad, P. N.; Kim, S.; Ohulchanskyy, T. Y.; Pudavar, H. E.; Pandey, R. K. *J. Am. Chem. Soc.* **2007**, *129*, 2669.
(44) Neves, M. G. P. M. S.; Serra, V. V.; Andrade, S. M.; Cavaleiro, J. A. S.; Costa, S. M. B. *New J. Chem.* **2010**, *34*, 2757.
(45) Simplicio, F. I.; Soares, R. R. D.; Maionchi, F.; Santin, O.; Hioka, N. *J. Phys. Chem. A* **2004**, *108*, 9384.
(46) Callot, H. J.; Johnson, A. W.; Sweeney, A. *J. Chem. Soc., Perkin Trans.* **1973**, *1*, 1424.
(47) Pangka, V. S.; Morgan, A. R.; Dolphin, D. *J. Org. Chem.* **1986**, *51*, 1094.
(48) Morgan, A. R.; Pangka, V. S.; Dolphin, D. *J. Chem. Soc. Chem. Commun.* **1984**, 1047.
(49) Meunier, I.; Pandey, R. K.; Senge, M. O.; Dougherty, T. J.; Smith, K. M. *J. Chem. Soc., Perkin Trans.* **1994**, *1*, 961.
(50) de Oliveira, K. T.; Silva, A. M. S.; Tome, A. C.; Neves, M. G. P. M. S.; Neri, C. R.; Garcia, V. S.; Serra, O. A.; Iamamoto, Y.; Cavaleiro, J. A. S. *Tetrahedron* **2008**, *64*, 8709.
(51) Tormena, C. F.; Lacerda, V.; de Oliveira, K. T. *J. Brazil Chem. Soc.* **2010**, *21*, 112.
(52) Corey, E. J.; Sarshar, S.; Lee, D. H. *J. Am. Chem. Soc.* **1994**, *116*, 12089.
(53) Redmond, R. W.; Gamlin, J. N. *Photochem. Photobiol.* **1999**, *70*, 391.
(54) Kuimova, M. K.; Botchway, S. W.; Parker, A. W.; Balaz, M.; Collins, H. A.; Anderson, H. L.; Suhling, K.; Ogilby, P. R. *Nat. Chem.* **2009**, *1*, 69.
(55) Uchoa, A. F.; Knox, P. P.; Turchielle, R.; Seifullina, N. K.; Baptista, M. S. *Eur. Biophys. J. Biophys.* **2008**, *37*, 843.
(56) Ogilby, P. R.; Foote, C. S. *J. Am. Chem. Soc.* **1982**, *104*, 2069.
(57) Hwang, T. L.; Shaka, A. J. *J. Magn. Reson., Ser B* **1993**, *102*, 155.
(58) Hyvarinen, K.; Helaja, J.; Kuronen, P.; Kilpelainen, I.; Hynninen, P. H. *Magn. Reson. Chem.* **1995**, *33*, 646.
(59) Baptista, M. S.; Indig, G. L. *J. Phys. Chem. B* **1998**, 4678.