Synthesis of chlorins and bacteriochlorins from cycloaddition reactions with porphyrins

Nuno M. M. Moura,* Carlos J. P. Monteiro, Augusto C. Tomé, M. Graça P. M. S. Neves, and José A. S. Cavaleiro*

LAQV-REQUIMTE, Chemistry Department, University of Aveiro, 3810-193 Aveiro, Portugal
Email: nmoura@ua.pt and jcavaleiro@ua.pt

Dedicated to Prof. Girolamo Cirrincione in recognition of his outstanding organic and medicinal chemistry contributions, on the occasion of his university retirement

Received mm-dd-yyyy Accepted mm-dd-yyyy Published on line mm-dd-yyyy

Dates to be inserted by editorial office

Abstract

Chlorins and bacteriochlorins are reduced porphyrin-type derivatives displaying characteristic structural, physical, and chemical features. Such features make chlorins and bacteriochlorins key “players” in several fields, and specifically in medicine as photosensitizers (PSs) for the diagnosis and treatment of neoplastic situations by photodynamic therapy. Cycloaddition approaches have become important synthetic tools to prepare chlorin and bacteriochlorin macrocycles. This review highlights the procedures developed over the last 10 years to synthesize chlorins and bacteriochlorins, namely through Diels–Alder and 1,3-dipolar reactions using porphyrin macrocycles as templates.

Keywords: Porphyrinoids, chlorins, bacteriochlorins, cycloaddition reactions, Diels–Alder, 1,3-dipolar cycloaddition

DOI: https://doi.org/10.24820/ark.5550190.p011.696
1. Introduction

Life on Earth would not exist as it is happening if natural processes such as respiration and photosynthesis would not be taking place. Porphyrin derivatives play a key role in such processes. Particularly in photosynthesis, the so-called chlorophylls are the most significant “actors”. This vital biological action has attracted the attention of many scientists along the centuries. In relation with this interest in understanding Nature and its events, many references can be put forward. Let us consider only three of such references which are specifically related to the scientists’ search for knowledge. One of them appeared in 1614 and was due to Sir Walter Raleigh in his “History of the World: Preface” (Man cannot give a true reason for the grass under his feet, why it should be green rather than red or any other color).\(^1\) Another reference was published in 1906 by Willstätter and relates the results obtained on the composition of chlorophyll,\(^2\) and the third one, a communication on the synthesis of chlorophyll a was published in 1960 by Woodward and 17 collaborators.\(^3\) Interestingly, thirty years later the full synthetic methodology related to the contents of that communication was published by the same authors.\(^4\)

The macrocycles of such compounds, in comparison with those of porphyrins, contain one reduced pyrrolic unit and so are dihydroporphyrin derivatives. All natural and synthetic compounds containing macrocycles of that type are called chlorins. In such way, the following question “How would be the life on Earth without the natural chlorins?” can be wavering in the mind of any human being.

There are other natural compounds named bacteriochlorophylls which occur in phototropic bacteria.\(^5\) Some of such compounds contain tetrahydro-type porphyrin macrocycles with two reduced pyrrole rings at opposite positions. Other derivatives isolated from bacterial enzymes contain two reduced pyrrole rings at adjacent positions and these are called isobacteriochlorins (Figure 1).
The vital functions played by such natural porphyrin derivatives certainly brought the motivation of many scientists to understand their synthesis and biosynthesis, mode of action and metabolism. That led to many studies and publications in the last century and synthetic routes for a significant variety of porphyrinoids and their potential applications have been put forward.\textsuperscript{6–10} Using porphyrin derivatives obtained by simple and efficient synthetic methods, applications have been demonstrated in several areas such as catalysis, materials, and medicine. Other applications as anticancer and anti-microbial agents are also very significant for humankind. Photodynamic therapy requires a good photosensitizing (PS) action taking place after its administration, accumulation in cancer cells and irradiation with an adequate radiation (\(\lambda > 630\) nm).\textsuperscript{11} The result is the formation of reactive oxygen species (mainly singlet oxygen and oxygen radicals) which bring death to the neoplastic cells. The absorption of radiation is then a key step in the process.\textsuperscript{12} In this sense, chlorins\textsuperscript{13} and bacteriochlorins\textsuperscript{14} are better sensitizers than porphyrins and isobacteriochlorins, since the former have higher absorptions at higher wavelengths in a wavelength range of deeper radiation penetration.\textsuperscript{15}

Certain chlorins and bacteriochlorins have already obtained medicinal approval and are being used in photodynamic therapy of cancer or are under clinical trials. Some examples will now be mentioned.

Foscan\textsuperscript{8}, the brand name of Temoporfin, a chlorin derivative, is being used since 2001 for lung, brain, neck and head cancer types (Figure 2).\textsuperscript{16,17}

Visudyne\textsuperscript{8}, the brand name of the chlorin derivative Verteporfin, formulated as a mixture of two isomers, is being used against age-related macular degeneration (Figure 3).\textsuperscript{17}

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{structures.png}
\caption{Structures of Porphyrin (a), Chlorin (b), Bacteriochlorin (c) and Isobacteriochlorin (d).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{temoporfin.png}
\caption{Temoporfin structure.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{verteporfin.png}
\caption{Verteporfin structure.}
\end{figure}
Figure 3. Structures of the two Verteporfin isomers.

In addition, other chlorin derivatives (e.g., MACE, Photochlor) and bacteriochlorins (e.g., Tookad) are still under clinical evaluation against several types of tumors (Figure 4).\textsuperscript{17–19}

Figure 4. Structures of MACE (a), Photochlor (b) and Tookad (c).

The potential use in several fields, mainly those with biological significance, have been a driving force for studies having hydroporphyrins as targets. As a result, many publications and book series have been published on this subject. Keeping in mind the potential applications of chlorins and bacteriochlorins, and considering the great synthetic capabilities brought by cycloaddition transformations, this review will deal with literature publications which appeared in the last 10 years on the synthesis of such porphyrin derivatives by cycloaddition procedures, although older ones may also be considered for a better understanding. Considering the structural features of the porphyrin macrocycles under study, their reactions as dienes, or as dienophiles or dipolarophiles will thus be discussed in this review.

2. Diels–Alder reactions

2.1 Vinyl-substituted porphyrins as dienes
Almost at the same time, the groups of Johnson\textsuperscript{20} and Inhoffen\textsuperscript{21} have shown that protoporphyrin-IX (Figure 5) can react in [4+2] cycloaddition processes with dienophiles like tetracyanoethylene (TCNE), dimethyl acetylenedicarboxylate (DMAD) or singlet oxygen. Chlorin-type compounds were the main products obtained
although in some cases the reactions gave mixtures of products, including chlorin, isobacteriochlorin, and other porphyrin-type derivatives.

During the last 10 years, interesting reviews covering the chlorins’ chemistry and their potential applications have been published.\textsuperscript{13,22} As far as the chlorins’ synthesis is concerned, several publications are mentioned in such reviews, mainly those involving the [4+2] cycloadditions with $\beta$-vinylporphyrins.

Oliveira \textit{et al}.\textsuperscript{13} have reported a concise, but very informative, review related with the natural occurrence and physical-chemistry features of chlorophylls $a$, $b$, $d$, $f$.

Figure 5). Information on synthetic methods, including the cycloaddition features leading to chlorins, is included in the review, as well as their potential applications in cancer photodynamic therapy and diagnosis, catalysis, and in the materials area.

![Figure 5. Structures of Protoporphyrin-IX and Chlorophylls $a$, $b$, $d$ and $f$.](image-url)
Taniguchi and Lindsey\textsuperscript{22} have published an excellent review about synthetic chlorins obtained from the derivatization of porphyrins. It covers the “chlorin world” until six years ago. Synthetic methods to afford chlorin-type macrocycles are put forward and the synthesis of more than 1000 chlorins or related macrocycles is considered. Spectral features of chlorins and chlorophylls are also compared and the possibility of synthetic chlorins becoming surrogates for chlorophylls is also discussed.

As far as the cycloaddition approaches are concerned, it can be stated that a very good survey until 2015 is considered in that review. It starts with the cycloaddition behavior of vinylporphyrins in the presence of oxygen and light. With protoporphyrin-IX there is formation of two isomeric photoprotoporphyrins (2a and 2b). The scission and rearrangement of the peroxo group in each compound leads to the formation of the corresponding isomeric chlorins 3a and 3b (Scheme 1). This method, leading to such chlorins and their protoporphyrin-IX precursors, was used in further studies carried out by several groups.\textsuperscript{21,23}

The initial cycloaddition method with protoporphyrin derivatives and TCNE and DMAD was extended to other dienophiles like urazines, maleimides, 4-nitro-1-nitrosobenzene and others with electron-deficient features. Chlorins have been the main products from these experiments, although in some cases the bis-addition took place resulting in the formation of isobacteriochlorins.\textsuperscript{22,23}

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{scheme1}
\caption{Chlorin products from the reaction of protoporphyrin-IX with \textsuperscript{1}O\textsubscript{2}.}
\end{scheme}

Brückner \textit{et al.} also have reviewed the synthetic procedures to bacteriochlorins and isobacteriochlorins.\textsuperscript{24} It is clear that the main products in each Diels–Alder cycloaddition depends on the starting vinylporphyrin and its reactivity.
For example, a spiro[chlorin–porphyrin]-type dimer was readily prepared by heating the [tetra-(β,β'-sulfoleno)porphyrinato]zinc(II) precursor in 1,2-dichlorobenzene at 140 °C for 2 h. The reaction underwent a [4+2] self-cycloaddition via a β,β'-bis(methylene)porphyrin intermediate generated in situ by thermal SO₂ extrusion, which in the absence of an added dienophile led to the formation of the spiro(chlorin–porphyrin zinc(II) complex 4 (Figure 6).²⁵

![Figure 6. Structure of the spiro(chlorin–porphyrin) 4.](image)

2.2 Porphyrins as dienophiles

The transformation of a porphyrin macrocycle into the related chlorin is of great synthetic significance. Can it be done by using the porphyrin macrocycle as a dienophile?

We have shown for the first time in 1997 that porphyrin macrocycles without vinyl substituents can react as dienophiles in the presence of reactive dienes which, in some cases, are generated in situ.²²,²⁶,²⁷ Usually chlorins are the main products obtained, but, depending on the physical-chemistry features of the starting porphyrin, other products such as bacteriochlorins or isobacteriochlorins can also be obtained. The initial studies in this area started with meso-tetraphenylporphyrin (TPP) 5 and the diene (ortho-benzoquinodimethane) generated in situ by thermal extrusion of SO₂ from benzosulfone 6 (Scheme 2). meso-Tetrakis(3-methoxyphenyl)porphyrin and meso-tetrakis(4-methoxyphenyl)porphyrin have also been used. The corresponding annulated chlorins like 7 have been the main isolated products. Starting with meso-tetrakis(pentafluorophenyl)porphyrin, not only the chlorin but also two stereoisomeric (cis and trans) bacteriochlorins 8a and 8b were isolated (Figure 7).²³
Scheme 2. [4+2] Cycloaddition of TPP with o-benzoquinodimethane.

Figure 7. Structures of the two obtained stereoisomeric bacteriochlorins 8a and 8b and the barrelene-chlorin 8c.

The method was extended to polyaromatic compounds considered to be stable dienes, such as pentacene and tetracene. The former gave rise to a barrelene-chlorin by cycloaddition across the pentacene 6,13 positions (8c, Figure 7); the latter, under microwave irradiation, gave rise to two isomeric chlorin derivatives. Herges et al. have applied this synthetic procedure for the synthesis of chlorins, bacteriochlorins and isobacteriochlorins. Isobenzofuran (IBF) was used as the diene and meso-tetraphenylporphyrin (TPP) 5, meso-tetrakis(pentafluorophenyl)porphyrin 9, and their Ni(II) complexes, as the dienophiles. Interestingly, these [4+2] cycloadditions took place at 70 °C.

Scheme 3. Depending on the amount of IBF used, the reaction with meso-tetrakis(pentafluorophenyl)porphyrin free base 9 gave, as the main product, the chlorin 10 or the bacteriochlorin 11; its Ni(II) complex gave the isobacteriochlorin Ni12 as the main or the only product.

Figure 8). In the reaction with the free base 9, the chlorin was the only product, while with the Ni(II) complex no product was isolated. Furthermore, the studied cycloadditions were claimed to be regio- and stereoselective.
Scheme 3. Cycloaddition of *meso*-tetraarylporphyrins with IBF.

Figure 8. Structures of bacteriochlorin (11) and isobacteriochlorin (Ni12).

The same group used the [4+2] method for the synthesis of borylated Ni(II) chlorins (Ni14a and Ni14b) and Ni(II) isobacteriochlorins Ni15 from IBF and Ni13, the Ni(II) complex of the corresponding borylated porphyrin (Scheme 4). When the free-base borylated porphyrin was used, the isolated products were the free-base chlorins and the bacteriochlorin.

Scheme 4. [4+2] Cycloaddition of borylated porphyrin Ni13 with IBF.
3. 1,3-Dipolar cycloadditions

3.1. Reaction of porphyrins with azomethine ylides

The possibility of porphyrins acting as dipolarophiles in 1,3-dipolar cycloadditions with azomethine ylides was reported for the first time by Cavaleiro and co-workers in 1999.\textsuperscript{31,32} The impact of this approach, to obtain high-value chlorins and other bis-adducts, is patent by the high number of scientific publications that appeared after the Cavaleiro’s seminal work and by the applications of the prepared compounds in different fields namely as catalysts,\textsuperscript{33–35} sensors, therapeutics for cancer, antimicrobial, antiviral or other biomedical applications.\textsuperscript{36}

In these pioneering studies, the azomethine ylide AZ1 was generated \textit{in situ} from the reaction of paraformaldehyde and \textit{N}-methylglycine (sarcosine) in refluxing toluene in the presence of TPP 5, meso-tetrakis(2,6-dichlorophenyl)porphyrin and meso-tetrakis(pentafluorophenyl)porphyrin 9. In general, as exemplified in Scheme 5 for porphyrin 9, the major products were the corresponding chlorin 16 and isobacteriochlorin 17. Although the bis-addition is highly site-selective to afford the isobacteriochlorins 17, the bacteriochlorin 18, and the tris-adduct 19 could also be obtained under more forcing conditions.\textsuperscript{32,37}

![Scheme 5](image.png)

**Scheme 5.** 1,3-Dipolar cycloaddition of \textit{meso}-tetrakis(pentafluorophenyl)porphyrin 9 and azomethine ylide AZ1.

In those earlier studies, the possibility to generate azomethine ylides using other amino acids (\textit{e.g.} glycine, \textit{L}-proline and \textit{trans}-4-hydroxy-\textit{L}-proline) or other aldehydes namely with sugar units, was also considered and \textit{N}-
H pyrrolidine-fused chlorins or a series of N- and C-glycoconjugated pyrrolidine-fused chlorins were prepared.\textsuperscript{32,38}

The extension of this approach to porphyrins of $A_3B$ type, bearing three pentafluorophenyl groups and one pyridyl unit at \textit{meso}-positions, was also successful, affording the expected chlorins,\textsuperscript{39} while in porphyrins with electron-withdrawing groups (\textit{e.g.} alkyloxycarbonyl substituents) at vicinal $\beta,\beta'$-pyrrolic positions, the cycloaddition occurred preferentially at the activated double bond affording “locked chlorins”.\textsuperscript{40,41}

A similar “locked chlorin” \textbf{21} was obtained from the reaction of $\beta$-octa(ethyloxycarbonyl)porphyrin \textbf{20} with azomethine ylide \textbf{AZ2} through a 1,3-dipolar cycloaddition (Scheme 6). The 1,3-dipole was generated \textit{in situ} by reaction of $N$-benzylglycine and trioxane in refluxing chlorobenzene affording chlorin \textbf{21} bearing a fused pyrrolidine unit in 18% yield. When the reaction was carried out in refluxing toluene it was observed that the yield of the reaction decreased by 8%.\textsuperscript{42}

\begin{equation}
\begin{aligned}
\text{Ph} & \quad \text{H} \quad \text{CO}_2\text{H.HCl} \\
\text{N} & \quad \text{O} \\
\text{EtO}_2\text{C} & \quad \text{CO}_2\text{Et} \\
\text{EtO}_2\text{C} & \quad \text{CO}_2\text{Et} \\
\text{N} & \quad \text{N} \\
\text{EtO}_2\text{C} & \quad \text{CO}_2\text{Et} \\
\text{EtO}_2\text{C} & \quad \text{CO}_2\text{Et} \\
\end{aligned}
\end{equation}

\begin{equation}
\begin{aligned}
\text{EtO}_2\text{C} & \quad \text{EtO}_2\text{C} \\
\text{N} & \quad \text{N} \\
\text{EtO}_2\text{C} & \quad \text{CO}_2\text{Et} \\
\text{EtO}_2\text{C} & \quad \text{CO}_2\text{Et} \\
\end{aligned}
\end{equation}

\textbf{Scheme 6.} 1,3-Dipolar cycloaddition of $\beta$-octa(ethyloxycarbonyl)porphyrin \textbf{20} with azomethine ylide \textbf{AZ2}.

The functionalization of $\beta$-octa(ethyloxycarbonyl)porphyrin scaffolds through 1,3-dipolar additions with bulky azomethine ylides exhibits two main drawbacks compared with \textit{meso}-tetraarylporphyrins. The first one is the difficulty to prepare the corresponding bacteriochlorin in appreciable amounts; the authors only observed the formation of the double-reduced derivative in very small amounts, even when the reaction was performed in the presence of large amounts of the 1,3-dipole precursors, leading to degradation of the reaction mixture. Under these conditions, the reaction of the Zn(II) complex of porphyrin \textbf{20} did not give rise to any reduced derivative, and only partial recovery of the starting material was possible. However, with the modification leading to chlorin \textbf{21} non-aggregation properties were demonstrated by the chlorin derivative; this feature, combined with the high fluorescence quantum yield and good singlet oxygen generation capability, makes chlorin \textbf{21} to be a good candidate for use in medicinal applications, such as a fluorescent marker and photosensitizer, opening the possibility for the compound to be exploited in theranostics.\textsuperscript{43}

The selection of \textit{meso}-tetraakis(pentafluorophenyl)porphyrin \textbf{9}, which contains four electron-withdrawing groups at \textit{meso} positions, as starting material for the preparation of chlorins through 1,3-dipolar cycloadditions, is not unexpected considering its high reactivity towards azomethine ylides. Additionally, further functionalization of the C\textsubscript{6}F\textsubscript{5} units through nucleophilic substitution of the \textit{para}-fluorine atoms\textsuperscript{9} with a range of
nucleophiles allowed the expansion of the library of this type of chlorins with improved features for PDT and other applications. Also, the presence of fluorine atoms makes these compounds amenable as probes for in vivo $^{19}$F NMR localization studies. Moreover, the incorporation of metals in the inner core of the reduced adducts opened new perspectives in terms of their applications in different fields as previously mentioned. For instance, in the context of PDT applications, Obata et al.\textsuperscript{44} selected chlorin 16 and the corresponding Pd(II) and Pt(II) complexes (Pd$^{16}$ and Pt$^{16}$) to evaluate the impact of the heavy atom effect on their performance as photosensitizers in PDT. The presence of a heavy atom on the photosensitizer molecule generally facilitates intersystem crossing and, consequently, the population of the triplet manifold, improving the singlet oxygen ($^{1}\text{O}_2$) quantum yield ($\phi_\Delta$), which is a key species in the PDT mechanism of action. The results showed that the $\phi_\Delta$ values increased in the following order: 16 (0.28) < Pd$^{16}$ (0.89) < Pt$^{16}$ (0.92) in C$_6$D$_6$ and their photocytotoxicity towards HeLa cells (light dose of 16 J·cm$^{-2}$ with $\lambda$ > 500 nm) increased in the same order (16 < Pd$^{16}$ < Pt$^{16}$) at a concentration of 0.5 µM. The study also showed that, upon photoirradiation, chlorins 16, Pd$^{16}$ and Pt$^{16}$, at a concentration of 5 µM, killed almost all cells, while the corresponding porphyrins showed no photocytotoxicity under the same conditions, possibly due to their poor light-absorbing properties.\textsuperscript{44}

The nucleophilic substitution of the para-fluorine atoms was also particularly relevant to open new therapeutic perspectives for chlorin 16, namely through the attachment of carboranyl groups,\textsuperscript{45} sugar units,\textsuperscript{46–49} among others. Considering the number of studies reported during the last decade, the relevance of the reaction of porphyrins with azomethine ylides is obvious.

In 2014, Oliveira et al.,\textsuperscript{50} looking for azomethine ylides precursors to perform the cycloaddition reactions under milder conditions,\textsuperscript{51,52} studied the reaction of 9, and its nickel complex Ni9, with the dipole AZ2 which was generated from precursor 22 with TFA in dichloromethane (Scheme 7). This precursor was obtained from the reaction of benzylamine and (chloromethyl)trimethylsilane, followed by reaction with aqueous formaldehyde in methanol. The best results were obtained with the Ni(II) complex and depending on the number of equivalents of 22, it was possible to obtain chlorin Ni23 or the isobacteriochlorins Ni24 and Ni25. It was also found that the free-bases (23–25) were easily obtained by demetallation in CH$_2$Cl$_2$/H$_2$SO$_4$.

\begin{equation}
\text{Scheme 7. Chlorin and isobacteriochlorins isolated from the reaction of azomethine ylide AZ2 with porphyrin 9 or its nickel(II) complex Ni9.}
\end{equation}

Under the context of biological applications, in 2014 Drain and co-workers\textsuperscript{53} reported a comprehensive study concerning the photophysical/photochemical properties (fluorescence quantum yield, singlet state lifetime, triplet quantum yield, two-photon absorption and quantum yield of singlet oxygen formation) of porphyrin 9,
and of the corresponding chlorin 16, isobacteriochlorin 17 and bacteriochlorin 18. Having in mind that glycosyl groups are able to target many cancer cell types because of their significantly increased number of glucose transporters and lectin-type receptors in the membrane, the authors extended the study to the corresponding non-hydrolysable thioglycosylated conjugates 26–29 (Figure 9) obtained through the nucleophilic substitution of the para-fluorine atoms with the adequate sugar unit.

Following the photophysical features found for the compounds under study, the authors came to the following conclusion: that the isobacteriochlorins 17 and 28 have a sufficient two-photon absorption (2PA) and emission to obtain high-quality two-photon microscopy images. These results also suggest that 28 is a good candidate for simultaneous imaging and PDT using near-IR light. Because of the significantly stronger Q bands at 730 nm and high triplet quantum yield, the glycosylated bacteriochlorin 29 has good potential as a PDT agent under single-photon conditions. In addition, the glycosylated chlorin 27 has photophysical properties that may be used for both detection and ablation of cancer. The compounds were also found to be photostable under experimental conditions and both 26 and 27 showed a notable capacity to inhibit head and neck squamous carcinoma xenograft tumor model in mice under standard light conditions.

![Figure 9](image_url)

**Figure 9.** Structures of porphyrin (26) and of the corresponding chlorin (27), isobacteriochlorin (28) and bacteriochlorin (29) functionalized with thioglycosyl units.

In 2016, Narumi *et al.*, considering that a PS with good water solubility can avoid cutaneous phototoxicity due to their rapid clearance from the body,\(^5^4\) reported the development of 32 bearing four maltotriose units (
The synthetic approach involved the reaction of chlorin 16 with the protected maltotriose 30 in the presence of diethylamine, followed by the deprotection reaction of the oligosaccharide moieties of 31 with sodium methoxide.  

The authors mentioned an improvement in the water-solubility of the resulting oligosaccharide conjugate when compared with previously reported monosaccharide counterparts with glucose units and a very high photodynamic activity towards HeLa cells (95% of killing at 3.0 μM, irradiation conditions λ > 610 nm for 30 min) with a IC₅₀ of approximately 1.3 μM for the selected assay system. The higher efficacy of the chlorin 32, when compared with the analogous porphyrin also synthesized, under identical irradiation conditions (reduction of cell viability to 35% at 5.0 μM) was also commented on. The high photodynamic activity of this water-soluble chlorin, attributed to its efficiency to generate ¹O₂, opens new perspectives for its use in vivo as an injectable PS.

Figure 10. Structures of chlorins 31 and 32 functionalized with maltotriose units.

In the same period, and also under the context of biological applications, chlorin 16 was selected as scaffold in the rational design of the third-generation photosensitizer 34 bearing galactodendritic units.  

Figure 11. The synthetic approach also involved the nucleophilic substitution of the para-fluorine atoms of the meso-tetrakis(pentafluorophenyl)chlorin 16 by galactodendritic units, giving 33, followed by the deprotection step of the sugar units, affording 34. The effectiveness of PS 34 was evaluated towards UM-UC-3 and HT-1376 bladder cancer cells, and the promising results obtained were in line with its photochemical and photophysical properties (like the ability to generate singlet oxygen, to interact with the proteins galectin-1 and human serum albumin (HSA) and to its high absorption in the red region of the electromagnetic spectrum). The results revealed that a single dose of light irradiation is necessary to induce high cytotoxicity towards UM-UC-3 bladder cancer cells, while for HT-1376 bladder cancer cells resistant to therapy, the photodynamic efficacy is improved after a second light irradiation treatment. This enhanced phototoxicity in HT-1376 cancer cells was justified by considering the ability of 34 to accumulate in the mitochondria, mediated by glucose transporter 1(GLUT1), in the period between single and repeated irradiation.
Figure 11. Structures of chlorins 33 and 34 functionalized with galactodendritic units.

In 2017, Drain and co-workers\textsuperscript{56} selected the NH chlorin 35, obtained from the reaction of 9 with paraformaldehyde and glycine (ylide AZ3), to obtain the chlorin derivative 36 and from this one the multifunctional chlorin platforms 37 and 38 appended with short polyethylene glycols [2-(2-methoxyethoxy)ethanol (O-PEG) and 2-(2-methoxyethoxy)ethanethiol (S-PEG) groups] and a carboxylate-linker (Scheme 8). This acid linker was considered to allow a rapid conjugation of the chlorins to bio targeting motifs such as proteins and oligonucleotides. As a proof of concept, the conjugation of derivatives 40 and 41 to a primary amine tethered on the 5' end of a 14 nucleotide (nt) single-strand DNA and to the exposed lysine amino groups on the lysozyme enzyme was reported, affording respectively derivatives 42–45 after activation of the carboxylic functionality with \textit{N}-hydroxysuccinimide. The possible uptake of the DNA–chlorin conjugate 43 by MDA-MB-231 breast cancer cells was evaluated and the results seemed to indicate that the aggregates observed at concentrations above 5 nM in/on the cells slowly disaggregate.

Later, the authors revisited the synthetic approach previously reported by Cavaleiro and co-workers\textsuperscript{32} to obtain the NH-chlorin 35 from paraformaldehyde and glycine (AZ3), and an improvement of its formation by using microwave heating was reported.\textsuperscript{57} During this endeavor, besides the expected NH-pyrrolidine, two dimers, and the same N-methyl chlorin obtained from the sarcosine ylide reaction were isolated. A mechanism based on the formation of the divergent N-(hydroxymethyl)-N-methylenemethanideaminium ylide was considered.

In 2020, an approach mediated by the divergent N-(hydroxymethyl)-N-methylenemethanideaminium ylide was extended to obtain chlorins 46 and 47 with amino and alkynyl linkers, thereby enabling other types of click chemistry for bioconjugation (}
In this work an optimized approach to obtain the NH pyrrolidine 35 from a dimeric chlorin precursor was reported. Also, synthetic access to Cu(II), Ni(II) and $^{64}$Cu complexes Cu48, Ni48 and $^{64}$Cu49, obtained from the previous reported chlorin 36 bearing the carboxyl functionality, was considered and new perspectives for their use in fluorescent imaging and positron emission tomography (PET) became available. This assumption was confirmed when the conjugates obtained from the free-base chlorin 36 and $^{64}$Cu-chlorin 49 with the peptide Hsp1a isolated from the venom of the Peruvian tarantula *Homeomma spec.*, were demonstrated to be excellent reporters to localize peripheral nerves by fluorescence and Cerenkov luminescence imaging.

![Figure 12](image_url) Figure 12. Free-bases and metal complexes of chlorins with amino, alkyne and carboxyl functionalities (46-49).

In 2019, Ol’shevskaya *et al.* selected chlorin 16 as a scaffold to obtain the maleimide-functionalized chlorin 51 and its Zn(II) complex Zn51 (Scheme 9). This approach considered the substitution of the para fluorine atoms of 16 by azide ions, followed by its reduction to chlorin 50. This chlorin was then converted into chlorin 51 by reaction with maleic anhydride. Although no biological activity has been reported yet, the authors commented that the presence of four maleimide groups in these chlorin derivatives could potentially improve their binding to proteins in living systems and consequently their biological activities.
Scheme 9. Synthetic approach to prepare maleimide-functionalized chlorins 51 and Zn51.

Considering that photodynamic therapy (PDT) can be an effective modality to treat several types of skin cancers, namely the most aggressive ones like melanoma, Castro et al. decided to revisit the reaction of 5,10,15-tris(pentafluorophenyl)-20-(4-pyridyl)porphyrin 52 with the azomethine ylide AZ1 and to evaluate the photodynamic action of the resulting chlorin 53 and isobacteriochlorin 54 (Figure 13) towards the melanotic cell line (B16F10 cells). For comparison, the studies were also extended to the starting porphyrin 52. The best PDT effect was obtained with chlorin 53 under red LED light irradiation (λ = 660 ± 20 nm, light dose of 5.4 J.cm⁻²) with cell death varying from 22.3% at a concentration of 0.39 μM to 83.3% and 94.8% for concentrations of 6.2 and 12.5 μM respectively. Under these irradiation conditions, 52 did not show any cytotoxicity while 54 showed only appreciable photocytotoxicity (72%) at concentrations higher than 12.5 μM. From the internalization studies, it was suggested that the subcellular localization of 53 and 54 mainly occurs in the mitochondria due to apoptosis being the main death pathway. Also, the ability of 53 to generate peroxynitrite (OONO⁻) under irradiation, prompted the authors to hypothesize that its high photocytotoxicity could be due to a synergistic effect involving the production of singlet oxygen and OONO⁻.

Figure 13. Structures of chlorin (53) and isobacteriochlorin (54) obtained from 5,10,15-tris(pentafluorophenyl)-20-(4-pyridyl)porphyrin (52).

Considering the recognized attractiveness of reduced-based PSs in cancer PDT, Mesquita et al. reported in 2021 the efficacy of the hydrophobic fluorinated porphyrin 9, chlorin 16 and isobacteriochlorin 17 (
Scheme 5), which were tested after being encapsulated in poly(vinylpyrrolidone) (PVP) micelles towards prostate cancer. The effectiveness of the nanoformulations obtained, namely 9@PVP, 16@PVP and 17@PVP as PS agents, was evaluated in vitro towards a PC-3 cell line and the results revealed that at concentrations of 20 μM, 16@PVP and 17@PVP were able to induce 53.1% and 87.9% of cell death respectively, after 4 h of incubation followed by 10 min of irradiation with red light (622 nm; irradiance of 17.6 mW.cm⁻²). The studies also confirmed the involvement of a caspase-dependent pathway in the apoptotic response, following the PDT treatment mediated by 16@PVP and 17@PVP. It was commented that the impressive emission properties of 17, combined with its high therapeutic action, open new perspectives for this formulation to be used as a theranostic agent. 

A recent study also showed that the pyrrolidine-fused chlorin of 5,15-bis(4-methyloxycarbonylphenyl)-10,20-bis-(pentfluorophenyl)porphyrin, incorporated into a metal–organic framework (MOF) functionalized with maltotriose, could efficiently target cancer cells with preferential uptake into pancreatic and breast cancer cell lines. 

The use of chlorins and isobacteriochlorins obtained from the reaction of porphyrins and ylides as PSs in the photoinactivation of microorganisms was also considered in different studies. In 2012, Costa et al., reported the synthesis and the photodynamic action of the pentacationic chlorin 55 towards two antibiotic-resistant bacterial strains, Staphylococcus aureus and Pseudomonas aeruginosa. The synthetic approach involved in the first step the reaction of chlorin 16 with 4-mercaptopypyridine, followed by alkylation of the neutral thiopyridylchlorin derivative with iodomethane. The antimicrobial photodynamic therapy (aPDT) treatments were performed with white light (400–800 nm) and red light (530–800 nm). The results were compared with the ones obtained with the cationic analog porphyrin and also with the well-known meso-tetrakis(1-methylpyridinium-4-yl)porphyrin tetra-iodide (TMPyP). These showed that chlorin 55 was the most effective PS for both antibiotic-resistant strains; for the Gram-positive bacterium S. aureus a reduction of 7.0 log units was observed after 5–10 min of irradiation, at a concentration of 0.5 μM, whereas for the Gram-negative P. aeruginosa, a similar photoinactivation occurred at 10 μM after 30 min of irradiation.

Figure 14) towards two antibiotic-resistant bacterial strains, Staphylococcus aureus and Pseudomonas aeruginosa. The synthetic approach involved in the first step the reaction of chlorin 16 with 4-mercaptopypyridine, followed by alkylation of the neutral thiopyridylchlorin derivative with iodomethane. The antimicrobial photodynamic therapy (aPDT) treatments were performed with white light (400–800 nm) and red light (530–800 nm). The results were compared with the ones obtained with the cationic analog porphyrin and also with the well-known meso-tetrakis(1-methylpyridinium-4-yl)porphyrin tetra-iodide (TMPyP). These showed that chlorin 55 was the most effective PS for both antibiotic-resistant strains; for the Gram-positive bacterium S. aureus a reduction of 7.0 log units was observed after 5–10 min of irradiation, at a concentration of 0.5 μM, whereas for the Gram-negative P. aeruginosa, a similar photoinactivation occurred at 10 μM after 30 min of irradiation.

Figure 14. Structures of the pentacationic chlorin (55) and of the corresponding zinc(II) complex (Zn55) functionalized with (1-methylpyridinium4-yl)sulfanyl moieties.

In 2014, Mesquita et al. evaluated the efficacy of the cationic chlorin 56 and the isobacteriochlorin 57 (
Figure 15) in the photodynamic inactivation of bioluminescent *Escherichia coli* (*E. coli*). These cationic derivatives were obtained by alkylation of the corresponding neutral derivatives 16 and 17 and the biological results showed that there is a direct relationship between the inactivation efficiency and the increasing number of charges on the molecules. The aPDT treatment in the presence of the dicationic isobacteriochlorin reached the limit of detection (6.1 log reduction) after a light dose of 36 J.cm\(^{-2}\) at 20 µM. For the neutral compounds and the cationic chlorin, non-significant inactivation was detected.

Soon afterwards, the same group found that the immobilization of the neutral chlorin 16 in 3-bromopropyl-functionalized silica and also in Merrifield resin-based materials provided promising photoactive materials towards *E. coli* (inactivation rates of ca 3.0 log at an irradiance of 4.0 mW.cm\(^{-2}\)) and that this bacterial reduction was maintained constant for at least three successive cycles of the aPDT treatment.\(^{66}\)

In 2019, Calmeiro et al.\(^{67}\) reported a similar methodology to the one reported in 2012 to obtain the free-base and the zinc(II) complex of the pyridinium chlorin derivatives 58 and Zn58 (Figure 16) and evaluated their efficacy to inactivate bioluminescent *E. coli*. The aPDT studies were also extended to the Zn(II) complex of the previously reported pentacationic chlorin Zn55 (Figure 14), which showed the best performance.

**Figure 15.** Structures of the cationic meso-tetrakis(pentafluorophenyl)chlorin (56) and isobacteriochlorin (57).

**Figure 16.** Pentacationic chlorin (58) and the zinc complex (Zn58) functionalized with 4-methoxy-1-pyridinium units.
In 2021, Lourenço et al.\textsuperscript{68} also selected chlorin 16 to prepare the cationic PS chlorin 60 bearing 4-(1H-pyrazol-3-yl)pyridinium groups and their aPDT efficiency was investigated against planktonic and biofilm forms of the Gram-negative bacterium \textit{E. coli} (Figure 17). For comparison, the authors also synthesized the cationic porphyrin analog 59. Although porphyrin 59 exhibited a higher antimicrobial efficiency against the planktonic cells of \textit{E. coli}, chlorin 60 showed to be more efficient towards biofilms. This photodynamic efficiency can be related to the higher levels of $^1$O$_2$ produced by 59, as well as with the higher absorption in the red region exhibited by 60.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure17.png}
\caption{Cationic porphyrin (59) and chlorin (60) bearing 4-(1H-pyrazol-3-yl)pyridinium groups.}
\end{figure}

In 2018, the \textit{meso}-tetrakis(pentafluorophenyl)porphyrin 9 was used by Durantini \textit{et al.}\textsuperscript{69} to prepare the pyrrolidine-fused chlorin 61 bearing a 4-(dimethylamino)phenyl residue. In this approach, the azomethine ylide AZ4 was obtained from 4-(dimethylamino)benzaldehyde and \textit{N}-methylglycine (Scheme 10). However, due to the Strecker degradation of \textit{N}-methylglycine to formaldehyde, AZ1 was also formed and chlorin 16 was isolated as a side product (28%). The photophysical properties of the new compound have been investigated and it was found that 61 is a fluorescence quencher; this is presumably due to the 4-(dimethylamino)phenyl substituent appended at the macrocycle. In addition, for the same compound, the triplet excited state was not detected under laser flash photolysis studies and as consequence, no singlet oxygen generation was detected by time-resolved phosphorescence detection.

Nevertheless, the authors found a different outcome when 61 was submitted to an acidic medium – under these conditions the protonation of the amino group was attained, promoting the formation of singlet and triplet states that opened the way to photophysical channels leading to fluorescence emission and singlet oxygen generation, respectively. This pH switch prompted the authors to investigate the photodynamic effect of 61 towards bacteria in acidic medium and the results showed a highly efficient eradication of \textit{E. coli}.

The fact that 61 can behave as an on/off molecular switch for the generation of singlet oxygen ($^1$O$_2$), i.e. by being activated in acidic media and deactivated in neutral media, can be an advantage for the eradication of bacteria in the stomach which has an acidic environment.
Scheme 10. Synthesis of 4-(dimethylamino)phenyl-pyrrolidine-fused chlorin 61.

Sobota et al. selected the ylide AZ1 to prepare chlorins 62 and 63 (Figure 18) via 1,3-dipolar cycloaddition from 2-nitroporphyrins bearing 4-(trifluoromethyl)phenyl or 3,5-bis(trifluoromethyl)phenyl substituents at meso positions. After confirming the efficacy of both chlorins to generate singlet oxygen, their antimicrobial photodynamic action was evaluated after being incorporated in lipid vesicles. The best outcomes were observed with chlorin 63 with log reduction of 4.84 for *Enterococcus faecalis* and 4.09 for *Staphylococcus aureus* at 1 μM; for *E. coli*, a reduction of 2.23 was only observed at 100 μM. No activity was found against the fungi *Candida albicans* and *Trichophyton mentagrophytes*. In a previous work, detailed structural analysis of the meso-aryl rings motion in related unsymmetrical pyrrolidine-fused chlorins, including 62 and 63, using NMR, UV spectroscopy and DFT theoretical calculations were reported.

![Figure 18](image-url) Structures of chlorins 62 and 63 obtained from the corresponding 2-nitroporphyrins.

In 2018, Silva et al. used the reaction of the azomethine ylide AZ1 with 5-(4-methoxycarbonylphenyl)-10,15,20-tris(pentafluorophenyl)porphyrin 64 to obtain the corresponding pyrrolidine-fused chlorin derivative 66 after hydrolysis of the ester group (Figure 19). A microwave irradiation protocol, using acetonitrile as solvent, was applied to obtain a series of metal complexes [Fe(III), Cu(II) and Zn(II)] from 64 and also the pyrrolidine-fused chlorin metal complexes from 66 (Fe66, Cu66, Zn66). The authors commented that the extension of this approach to N-methyl nitro, to afford isoxazolidine-fused chlorins 65, provided lower yields and selectivity’s (when compared with azomethine ylide as 1,3-dipole) with the disadvantage of a retro-cycloaddition reaction occurring to the isoxazolidine-fused chlorins during the acidic hydrolysis of the ester group.
Figure 19. 5-(4-Methoxycarbonylphenyl)-10,15,20-tris(pentafluorophenyl)porphyrin (64), isoxazolidine-fused chlorins (65a,b) and the pyrrolidine-fused chlorin derivatives (66) and Fe, Cu, Zn complexes.

In 2021, the same group,73 reported the extension of the protocol to obtain the N-methylpyrrolidine-fused meso-tetrakis(4-carboxyphenyl)chlorin 68, from the symmetric meso-tetrakis(4-methoxycarbonylphenyl)-porphyrin 67 (Figure 20). The incorporation of this chlorin into a nanostructured porous TiO₂ matrix afforded a new material with high sensing efficiency able to detect the explosive triacetone triperoxide in the gas phase; this explosive is considered to be even more dangerous than the powerful nitroaromatics due to its extreme sensitivity to detonation upon impact, heating, or friction.

Figure 20. Structure of chlorin 68 obtained from 1,3-dipolar cycloaddition of AZ1 with porphyrin 67 followed by hydrolysis of methyl ester units.

Herges et al.,74 reported that the square-planar chlorin and isobacteriochlorin Ni(II) complexes (Ni16 and Ni17), and also the starting porphyrin Ni9, accomplish the preconditions for the design of efficient spin-switching systems (from S = 0 to S = 1); that is due to their ability to coordinate with axial ligands, thus affording the required square-pyramidal and octahedral complexes (Scheme 11).

The capability of the Ni(II) complexes Ni16 and Ni17 to act as spin-state switches (in the presence of pyridine the metal spin-state changes and the Ni(II) is able to axially coordinate pyridine molecules) was scrutinized by UV–vis spectroscopy and NMR titration experiments using pyridine as ligand. The results showed that the association constants K₁ and K₂ (determined by NMR spectroscopic titration) increase in the following order: porphyrin (Ni9) < chlorin (Ni16) < isobacteriochlorin (Ni17). With these findings it could be concluded that the
Ni(II) complexes Ni16 and Ni17 are good platforms for the coordination-induced spin-state switching (CISSS) and are potential candidates to be used as contrast agents in the field of medical imaging and interventional radiology.

Under the context of molecular spin switches' applications, Ikeue et al. extended the method of Herges and prepared the stable diamagnetic (S = 0) Ni(II) complex of pyrrolidine-fused pyrrocorphin Ni19 (see Scheme 5). The free-base of this hexahydroporphyrin was previously reported by Cavaleiro and co-workers and contrary to other pyrrocorphins it demonstrated to be less air sensitive.

The impact of the ligand in electronic features of the Ni(II) complex Ni19 was also evaluated in the presence of pyridine. The addition of an excess of pyridine to Ni19 in toluene solution yielded first the mono-pyridine five-coordinate complex and then the bis-pyridine six-coordinate paramagnetic complex (S = 1). These compounds were characterized by employing several techniques as UV-Vis and 1H NMR spectroscopies, cyclic voltammetry, X-ray analysis and DFT calculations.

The comparison of the binding constants obtained for the Ni(II) pyrrocorphin (Ni19) with the ones reported by Herges et al. for the Ni(II) complexes of the corresponding porphyrin (Ni9), chlorin (Ni16), and isobacteriochlorin (Ni17) confirmed that the highest reduction of the porphyrin core is associated with a better performance in the desired coordination-induced spin-state switching (Ni9 < Ni16 < Ni17 < Ni19).

3.2 Reaction of porphyrins with nitrile oxides

Ostrowski et al. reported that the reaction of TPP with alkyl nitrile oxides NO1 afforded isoxazoline-fused chlorins 69.

Scheme 12. Nitrile oxides NO1 were generated in situ from unfunctionalized or functionalized nitroalkanes and a dehydrating agent (PhNCO or (Boc)2O) in the presence of an organic base (NEt3 or DABCO). The yields
were low to moderate (most of the starting TPP was recovered) and best results were obtained using functionalized nitroalkanes. Chlorins 69a–d were isolated in 24–63% yield considering the recovered TPP.

![Scheme 12](image)

Scheme 12. Isoxazoline-fused chlorins 69 synthesized by reaction of TPP 5 and nitrile oxides.

The reaction of meso-tetra-2-thienylporphyrin (70) and meso-tetrakis(5-nitro-2-thienyl)porphyrin (71), both as free-bases and Zn(II) complexes, with 2,6-dichlorobenzonitrile oxide under various experimental conditions (Scheme 13), was studied by Oliveira et al. The obtained products were the new chlorin derivatives 72, 73 and their Zn(II) complexes Zn72 and Zn73. The results showed that in these reactions the free-bases 70 and 71 were better dipolarophiles than the corresponding Zn(II) complexes and confirmed that the nitro derivative 71 was more reactive than 70, affording the two regioisomeric bisadducts 74 and 75, while no bisadducts were formed from 70.
Scheme 13. 1,3-Dipolar cycloaddition of (2-thienyl)porphyrins with 2,6-dichlorobenzonitrile oxide.

Feng et al.\textsuperscript{78} reported the reaction of an AB\textsubscript{3}-type porphyrin with 2,6-dichlorobenzonitrile oxide and obtained a mixture of the four expected site- and regio-isomeric monoadducts (illustrated with structure 76a) and the bacteriochlorin bisadduct 77 (Figure 21). The five products (each obtained in ca. 10\% yield) were separated by column chromatography on silica gel. Metalation and alkaline hydrolysis of the ester group of the four monoadducts afforded the corresponding Ni(II) porphyrin carboxylic acids (illustrated with structure 76b) which were then used in dye-sensitized solar cells. The highest conversion efficiency of 3.65\% with a short circuit photocurrent density of 8.55 mA/cm\textsuperscript{2}, an open-circuit voltage of 610 mV, and a fill factor of 0.70 was obtained for one of the isomers.
3.3. Reaction of porphyrins with silyl nitronates

Isoxazoline-fused chlorins of type 79 are typically prepared from the 1,3-dipolar cycloaddition of porphyrins with nitrile oxides, as shown in the previous section. However, considering that the yields of those reactions are frequently low, Senge et al.\textsuperscript{79} studied an alternative strategy to obtain isoxazoline-fused chlorins from porphyrins and silyl nitronates (SN). Silyl nitronates are 1,3-dipoles that behave as synthetic equivalents of nitrile oxides. As shown in

Scheme 14, meso-tetrakis(pentafluorophenyl)porphyrin (9) reacted with silyl nitronates SN1a–c (generated from the corresponding aliphatic nitro compounds and triisopropylsilyl chloride) to give, directly, the isoxazoline-fused chlorins 79a–c due to the spontaneous elimination of silanol from the intermediate N-silyloxyisoxazolidine cycloadducts 78a–c. The obtained yields of the propyl and pentyl derivatives were quite low (16\% and 18\%, respectively), but the methyl ester derivative 79c was obtained in a reasonable yield (34\%). No bisaddition was observed in these reactions. Similar reactions using the less electron-deficient meso-tetrakis(3,5-difluorophenyl)porphyrin as dipolarophile yielded only traces of the desired chlorin, while with TPP no product was detected.

3.4. Reaction of porphyrins with nitrones

The 1,3-dipolar cycloaddition of porphyrins with nitrones gives isoxazolidine-fused chlorins, useful compounds for a range of applications, namely for PDT. An interesting study showed that the isoxazolidine-fused chlorins are also useful precursors to other porphyrin derivatives bearing extended exocyclic systems, as illustrated in Scheme 15.

Scheme 15. The isoxazolidine-fused chlorin 80, prepared from the reaction of meso-tetrakis(pentafluorophenyl)porphyrin (9) with N-methyl nitrone N1 (generated in situ from N-methyl hydroxylamine hydrochloride and paraformaldehyde), was submitted to a microwave-assisted palladium-catalyzed hydrogenation transfer with cyclohexene that led to the cleavage of the isoxazolidine N–O bond (Scheme 15). The initially formed secondary amine function that resulted from the isoxazolidine ring-opening underwent a spontaneous nucleophilic aromatic substitution of the o-F atom of the adjacent aryl ring and led to the mono-annulated chlorin 81 in 32% yield. Further treatment of 81 with NaH induced a second intramolecular nucleophilic aromatic substitution generating the bis-annulated chlorin 82. The final chlorin derivative, that comprises a 2,3-dihydro-1H-benzo[b]azepine ring system and a 2H-pyran ring, exhibited an unusual asymmetry and displayed a strong absorption band centered at 664 nm.
Scheme 15. Synthesis of isoxazolidine-fused chlorin 80, and further intramolecular nucleophilic aromatic substitution to afford mono- (81) and bis-annulated chlorins (82).

A recent study showed that the yields of the isoxazolidine-fused chlorins 84 vary greatly with the nature of the meso-aryl groups of the starting porphyrin. While the reaction of meso-tetrakis(pentafluorophenyl)porphyrin (9) with N-methyl nitrone N1 afforded chlorin 84b in 71% yield, similar reactions with meso-tetraphenylporphyrin (5) and meso-tetrakis(2,6-dichlorophenyl)porphyrin (83) gave the corresponding chlorins 84a and 84c in 22% and 20% yield, respectively (Scheme 16).

Scheme 16. Reaction of meso-tetraarylporphyrins and N-methyl nitrone N1 afforded isoxazolidine-fused chlorins
The same study also showed that the yields of the isoxazolidine-fused chlorins 85 vary greatly with the nature of the N-substituent group at the nitro, being the highest yield (91%) for R = cyclohexyl (Scheme 17).\(^2\) Using the most reactive porphyrin (9) and the most reactive nitrones, bacteriochlorin-type bisadducts 86 and 87 were also obtained, albeit in low yields (Scheme 17).

\[ \text{Scheme 17. Chlorins 85 and bacteriochlorins 86 and 87 synthesized by reaction of porphyrin 9 with various nitrones.} \]

The synthesis of mixed bisadducts was also considered in the previous study.\(^2\) The obtained experimental results confirmed that the selectivity of the bisaddition reactions can be set to provide selectively isobacteriochlorins or bacteriochlorins simply by reversing the sequence of reactions of a porphyrin with two different 1,3-dipolar species. In fact, the reaction of porphyrin 9 with nitrone N1, followed by the reaction of resulting chlorin 85a with azomethine ylide AZ1 gave the isobacteriochlorins 88 and 89 (as a mixture of the four possible isomers) (Scheme 18). However, the initial addition of the azomethine ylide to porphyrin 9 followed by the addition of nitrone N1 to the resulting chlorin 16, led to the selective formation of bacteriochlorins 90 (Scheme 19).
3.5. Reaction of porphyrins with nitrile imines

The reactions of porphyrins with nitrile imines leads to pyrazoline-fused chlorins. This type of reaction was studied using porphyrin 9 and nitrite imines Ni1 (generated in situ from functionalized α-chlorohydrazone derivatives and NEt3 or DABCO) under various experimental conditions. Unfortunately, the expected pyrazoline-fused chlorins 91 were obtained in low yields (Scheme 20). In these reactions, porphyrin derivatives 92 were also formed and, in some cases, they are the main products. Best yields for cycloadducts 91 were obtained when Ar = Ph and R = Ph, 4-MeC6H4 or 4-MeOC6H4 (29%, 32% and 19% yield, respectively). The presence of an α-ethyl group on the N-aryl ring (Ar = 2-EtC6H4) led to a decrease of the yield of 91 to 7%, probably due to steric hindrance. In those cases, the formation of derivatives 92 prevail, being isolated in 43% (R = 4-MeC6H4) or 32% yield (R = 4-MeOC6H4). On the other hand, nitro substituents at the N-aryl ring suppress the reaction – no products were isolated when Ar = 4-NO2C6H4 or 2,4-(NO2)2C6H3. Of note is that pyrazoline-fused chlorins 92...
display a strong absorption band at long wavelengths ($\lambda_{\text{max}} \approx 660 \text{ nm}$) and may be useful dyes for biomedical applications.

Scheme 20. Reaction of meso-tetrakis(pentafluorophenyl)porphyrin 9 with nitrile imines.

4. Other cycloadditions

4.1. Reaction of porphyrins with diazafulvenium methides

The diazafulvenium methide DM1, generated by thermal extrusion of SO$_2$ from 2,2-dioxo-1H,3H-pyrazolo[1,5-c][1,3]thiazole 94, reacts with meso-tetraarylporphyrins 93a-h in [8π+2π] cycloadditions to give 4,5,6,7-tetrahydropyrazolo[1,5-a]pyridine-fused chlorin derivatives 95a-h (Scheme 21).$^{85}$ The yield of these reactions is low or moderate, but most of the starting porphyrin is recovered. The reaction of chlorins 95a-d with sulfone 94 in refluxing 1,2,4-trichlorobenzene afforded the corresponding bacteriochlorins 96a-d. The best yield (36%) was obtained for bacteriochlorin 96b, that was isolated as a single isomer with cis configuration (determined by X-ray crystallography). Chlorins 95 and bacteriochlorins 96 exhibit absorption spectra with a long wavelength absorption band with a maximum at ca. 649 nm and at ca. 727 nm, respectively. Compounds of these types are thus potentially useful for biomedical applications.
It has been recently shown that chlorins 97 and bacteriochlorins 98 are efficient photosensitizers for the PDT of cancer. In vitro PDT studies with the luminescent platinum(II) chlorin 97 showed that it is active against human melanoma, esophageal adenocarcinoma and bladder carcinoma cell lines and in vivo studies using a A375 melanoma mouse model proved that this chlorin is able to impair tumor growth and, importantly, allows for image guided cancer treatment. Chlorin 98 also revealed to be an efficient PS against human melanoma and esophageal carcinoma cell lines, with IC50 values in the nanomolar range (13 nM and 27 nM, respectively).
Figure 22. Structures of platinum(II) chlorin 97 and free base chlorin 98.

5. Porphyrinoids cycloaddition reactions

The preparation of chlorin- and (iso)bacteriochlorin-like derivatives of other porphyrinoids has been receiving special attention from synthetic chemists due to their electronic, optical, and photophysical properties and potential applications such as photosensitizers in PDT. Some examples are described below.

The 2-methyl-N-confused porphyrin reacted with an excess of 2,6-dichlorobenzonitrile oxide NO2 in refluxing benzene for 5 h to afford as main products a mixture of four monoadducts (29%) and one bis-adduct (20%) (Scheme 22). The reaction exhibited neither stereo- nor regioselectivity for the chlorin-type derivatives as the 2-aza-21-carbachlorins were obtained in a 55:27:13:5 molar ratio for 100a:100b:100c:100d, respectively. However, the addition of a second 2,6-dichlorobenzonitrile oxide unit to the N-confused macrocycle allowed for the stereo- and regioselective synthesis of the bacteriochlorin-type derivative 101 bearing two isoxazoline-fused moieties.87

It is worth mentioning that the use of 2-N-methyl confused porphyrin 99 inhibits the dynamic process, which leads to the formation of two different N-confused tautomers due to the π-electron delocalization on the macrocycle, and consequently prevents the cycloaddition reaction at the pyrrolic positions in trans-position with respect to the N-confused pyrrole.

Scheme 22. Synthesis of 2-aza-21-carbachlorins 100a–d and the corresponding bacteriochlorin 101 by reaction of 2-methyl-N-confused 99 porphyrin with nitrile oxide NO2.

Porpholactones are porphyrin analogs in which one or more of the β,β'-bonds have been replaced by a lactone unit through oxidation of the corresponding porphyrin derivative.88 Hydroporpholactones can be prepared by reduction of β-pyrrolic positions with hydrazines. Treatment of the phenyl-meso-substituted porpholactone 102 with hydrazine gives the chlorin-like derivative with reduction of the opposite position...
concerning the lactone-type unit. On the other hand, the treatment of the meso-(pentafluorophenyl)-substituted analog with Woollins’ reagent and PhMe$_2$SiH affords the reduction of one of the adjacent positions.

The reaction of porpholactone 102 with OsO$_4$ in CHCl$_3$ affords almost in quantitative yields the chlorin-type derivative 103 due to the osmylation reaction at the opposite pyrrolic position considering the pyrrolinone unit (Scheme 23). When the reaction was carried out with the Zn(II) or Pt(II) complexes of porpholactone 102 the transformation had taken place at the two pyrrole-type units adjacent to the oxazolone moiety. The corresponding metallo isobacteriochlorin-like osmate ester derivatives were isolated as an inseparable mixture in an approximate ratio of 2:1 in a 75% yield.

Scheme 23. Synthetic approaches to prepare hydoporpholactones.

It should be appreciated that the free base and metallo osmate ester derivatives are valuable intermediates to prepare bacteriodilactones and metalloisobacteriodilactones.

Tomé and co-workers studied the reactivity of free base porpholactone 104 with azomethine ylides and nitrones. The reaction with azomethine ylides (generated in situ from the reaction of paraformaldehyde with $N$-methylglycine or $N$-benzylglycine) afforded the corresponding mono-adducts 105a or 105b in 85% and 43%, respectively (Scheme 24). The reaction is site-selective and occurs at an adjacent position of the lactone-like unit, affording new compounds with isobacteriochlorin-type electronic features.

The reaction with nitrones (generated in situ by basic treatment of $N$-methylhydroxylamine hydrochloride or $N$-benzylhydroxylamine hydrochloride and paraformaldehyde) also displays site-selectivity. However, the regioisomers 106 and 107 were formed from the cycloaddition reaction at the pyrrolic unit opposite to the lactone moiety in yields ranging from 8% to 22% (Scheme 24).
Scheme 24. 1,3-Dipolar cycloadditions of porpholactone 104 with azomethine ylides and nitrones.

Zhang et al.\textsuperscript{94} used 108 to prepare the cis- and trans-porphodilactone bacteriochlorin-like derivatives 109a,b in 57% and 66% yield, respectively, using the 1,3-dipolar cycloaddition approach with azomethine ylide prepared from sarcosine and paraformaldehyde (Scheme 25).

Scheme 25. Synthesis of porphodilactones bacteriochlorin-like 109a,b.

Lee and co-workers\textsuperscript{95} extended the 1,3-dipolar cycloaddition approach of azomethine ylides to prepare Pd(II) complex (benz)chlorin 111 from the reaction of the precursor 110 and the azomethine ylide generated \textit{in situ} at 90 °C in toluene for 1 h under N₂. The bacteriochlorin-type derivative 112 was also isolated as a diastereomeric mixture in 39% yield, being obtained as the chlorin-type analog but only in vestigial amounts. However, the authors found that the cycloaddition reaction, as well as the products formed, are temperature
dependent. By reducing the reaction temperature to 80 °C, it allowed for the mono-adduct to be isolated in comparable yield (37%) to the one obtained for the bis-adduct when the reaction was carried out at 90 °C (Scheme 26).

Scheme 26. 1,3-Dipolar cycloaddition of Pd(II) complex (benzi)porphyrin 110 and azomethine ylide.

The oxidation of the chlorin-type derivative 111 with DDQ afforded the corresponding π-extended N-methylpyrrole-fused benzoporphyrin 113. Nevertheless, attempts to perform the oxidation of the bacteriochlorin-type derivative 112 were unsuccessful, leading to decomposition of the starting porphyrinoid (Scheme 26).

The same research group used a similar synthetic approach to prepare analogous pentaphyrin chlorin-type derivatives. The azomethine ylide (prepared in situ by reaction of sarcosine and paraformaldehyde) reacted with meso-diethylmalonylidene-(m-benzi)pentaphyrin affording the pentaphyrin chlorin-type analog 114 in 49% yield.

Figure 23.95 After an oxidation step of the pyrrolidine ring, followed by reduction of the macrocycle with NaBH₄, the resulting product was submitted to another 1,3-dipolar cycloaddition under the same conditions to afford the chlorin-type derivative 115 in 75% yield. The π-system delocalization can potentially be improved by submitting compound 115 to a new sequential oxidation and reduction protocol, affording a new derivative bearing two fused pyrrole-type moieties.
These synthetic approaches also show the usefulness of the 1,3-dipolar cycloaddition procedure and synthesis of chlorin-type derivatives as templates to prepare π-extended porphyrinoids with improved photophysical properties.

The N-methyl-5,15-diazaporphyrinium derivative 116 reacted via a Diels-Alder reaction with a large excess of cyclopentadiene to afford regio- and stereoselectively the bis-adduct 117 in 53% yield (Scheme 27). The preparation of N-methyl-5,15-diazaporphyrinium cations significantly enhances the reactivity of porphyrin-related macrocycles as dienophiles through the substantial decrease of LUMO energy thereby improving the match with the HOMO energy level of cyclopentadiene.96

**Figure 23.** Structures of (m-benzi)pentaphyrin chlorin-type derivatives 114 and 115.

**Scheme 27.** Diels-Alder reaction of N-methyl-5,15-diazaporphyrinium cation 116 and cyclopentadiene.

### 6. Conclusions

This review covers the last ten years’ literature data on the synthetic applicability of cycloaddition reactions to modify the structures of easily available porphyrinic macrocycles and making available other potential significant reduced porphyrin and porphyrin-type derivatives. The cycloaddition approach is a valuable tool in synthetic chemistry. Its application in the porphyrin field gives rise to novel reduced derivatives, namely chlorins and bacteriochlorins, which might demonstrate suitable and improved properties to be applied in several areas, including mainly in medicine as photosensitizers for the cancer photodynamic therapy. The recent development of cationic chlorins also allows for the consideration of photodynamic processes in the photoinactivation of microorganisms, including antibiotic resistant ones.
The versatility and usefulness of the cycloaddition reactions, particularly the 1,3-dipolar cycloadditions, has allowed a new synthetic route leading to dihydro- and tetrahydro-type derivatives where chlorins and bacteriochlorins, bearing substituents with different electronic behaviors in comparison with the starting porphyrin macrocycles, have a special significance.

The cycloaddition approach was also successfully extended to porphyrin-related macrocycles to prepare chlorin- or bacteriochlorin-like analogs with different features. Additionally, the new reduced compounds can be used as templates to prepare oxidized analogs, thus providing systems with π-system delocalization properties. It can be concluded that the development of new chlorins and bacteriochlorins with improved structural features and properties is a requirement to overcome the challenges brought by the several fields, mainly medicine, where such groups of novel derivatives provide avenues for potential applications.

Acknowledgements

Thanks are due to the University of Aveiro and Fundação para a Ciência e a Tecnologia (FCT) for the financial support to LAQV-REQUIMTE UIDB/50006/2020) through national funds and, when applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement, and to the Portuguese NMR Network. NMM Moura thanks his research contract (CDL-CTTR-88- 89-97-ARH/2018) which is funded by national funds (OE), through FCT, in the scope of the framework contract foreseen in numbers 4, 5 and 6 of article 23, of the Law Decree 57/2016, of August 29, changed by Law 57/2017, of July 19. CJP Monteiro thanks the financial support of his research contract to the FCT project PREVINE (FCT-PTDC/ASP-PES/29576/2017).

References


https://doi.org/10.3762/bjoc.15.263

https://doi.org/10.1021/acsabm.1c00218

https://doi.org/10.1016/j.jphotobiol.2021.112301

https://doi.org/10.1021/acsabm.0c01324

https://doi.org/10.1039/c2pp25113b


https://doi.org/10.1016/j.dyepig.2014.04.025

https://doi.org/10.1016/j.dyepig.2019.03.021

https://doi.org/10.1016/j.dyepig.2021.109557

https://doi.org/10.1002/chem.201800060

https://doi.org/10.1016/j.dyepig.2018.08.004

https://doi.org/10.1039/C7RA02217D

https://doi.org/10.1039/C7NJ05165D

https://doi.org/10.1016/j.dyepig.2021.109721
[https://doi.org/10.1021/acs.inorgchem.5b01756](https://doi.org/10.1021/acs.inorgchem.5b01756)

[https://doi.org/10.1016/j.jinorgbio.2017.10.012](https://doi.org/10.1016/j.jinorgbio.2017.10.012)

[https://doi.org/10.3987/COM-11-12347](https://doi.org/10.3987/COM-11-12347)

[https://doi.org/10.1002/ejoc.201402227](https://doi.org/10.1002/ejoc.201402227)

[https://doi.org/10.1016/j.dyepig.2013.01.013](https://doi.org/10.1016/j.dyepig.2013.01.013)

[https://doi.org/10.3987/COM-10-12125](https://doi.org/10.3987/COM-10-12125)

[https://doi.org/10.1016/S0040-4039(01)02243-2](https://doi.org/10.1016/S0040-4039(01)02243-2)

[https://doi.org/10.1039/C5OB00800J](https://doi.org/10.1039/C5OB00800J)

[https://doi.org/10.1039/G6Q00771F](https://doi.org/10.1039/G6Q00771F)

[https://doi.org/10.3998/ark.5550190.0011.504](https://doi.org/10.3998/ark.5550190.0011.504)

[https://doi.org/10.1246/bcsj.20110408](https://doi.org/10.1246/bcsj.20110408)

[https://doi.org/10.1039/D0MD00433B](https://doi.org/10.1039/D0MD00433B)


[https://doi.org/10.1021/ko3000817](https://doi.org/10.1021/ko3000817)

[https://doi.org/10.1002/adsc.201200720](https://doi.org/10.1002/adsc.201200720)


https://doi.org/10.1039/c3ob40138c


https://doi.org/10.1021/acs.joc.8b02628


https://doi.org/10.1021/acs.accounts.9b00119


https://doi.org/10.3390/molecules25112642


https://doi.org/10.1039/C9CP01177C


https://doi.org/10.1039/C4CC04283B


https://doi.org/10.1002/chem.201905402

This paper is an open access article distributed under the terms of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/)