This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this Accepted Manuscript with the edited and formatted Advance Article as soon as it is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal’s standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
Isoxazolidine-fused meso-tetraarylchlorins as key tools for the synthesis of mono- and bis-annulated chlorins†

António Aguiar,Andreia Leite, André M. N. Silva, Augusto C. Tomé, Luís Cunha-Silva, Baltazar de Castro, Maria Rangel and Ana M. G. Silva

The microwave-assisted catalytic hydrogenation of the isoxazolidine-fused meso-tetrakis(pentafluorophenyl)chlorin afforded directly a mono-annulated chlorin with a singular 1-methyl-2,3-dihydro-1H-benzo[b]azepine ring that resulted from the cleavage of the isoxazolidine N-O bond followed by an intramolecular nucleophilic aromatic substitution of an o-F atom. The subsequent treatment of the mono-annulated chlorin with NaH induced a second intramolecular nucleophilic aromatic substitution, generating a bis-annulated chlorin having an additional 2H-pyran ring.

The optical properties, coordination chemistry and redox behaviour of porphyrins have been successfully modulated through modifications in the porphyrin frame. The insertion of exocyclic ring systems fused to the periphery of the macrocycle represents one of these successful examples that have been widely employed to reach porphyrinic macrocycles exhibiting extended π-electron delocalization and distortions of planarity. Typically, these macrocycles are endowed with large red-shift absorption spectra, pointing for potential applications in photodynamic therapy (PDT) and as models of naturally occurring compounds, as oxidation catalysis and as chromophores in molecular recognition and materials science.

The electronic properties of the porphyrin macrocycle can also be modulated through the establishment of β-to-meso linkages yielding annulated macrocycles, holding a greater coplanar conformation. An increasing number of annulated porphyrins containing β-to-meso-aryl linkages have been developed to date, whether by introducing a direct β-to-o-phenyl linkage forming a five-membered ring or by using heteroatoms or carbonyl functions as linkers forming six-membered ring systems. In an earlier example, Boyle et al. prepared a meso-(2-iodophenyl) substituted porphyrin, which after treatment with a palladium(0) complex, in the presence of base, led to C–C coupling and to an additional five-membered fused ring (A, Figure 1). More recently, Brückner et al. described the preparation of mono- and bis-chromene-annulated meso-(pentafluorophenyl)chlorins (B, Figure 1) by intramolecular aromatic substitution of o-F atoms at a meso-pentafluorophenyl-2,3-dihydroxychlorin. There are also interesting but rare examples involving the formation of seven-membered ring systems. In one of these examples, Scott et al. presented distorted porphyrins with two exocyclic naphthocycloheptanones, which after oxidative dehydrogenation gave almost perfectly planar porphyrins bearing bis(naphthoazulenone) ring systems (C, Figure 1), exhibiting absorption bands at unusual wavelengths (> 850 nm).

On the other hand, annulation can be achieved through modification of naturally occurring macrocycles or via de novo synthesis, yielding a variety of exocyclic rings. Examples of such analogues are chlorins with fused quinoxaline and benzimidazole rings (D and E, Figure 1) and 132,173-cyclophosphorbid enol (F, Figure 1).

Figure 1. Examples of annulated porphyrins and chlorins containing β-to-meso linkages.
Among the wide number of transformations that can be performed in the porphyrin frame, the 1,3-dipolar cycloadditions (1,3-DC) offer a very useful method for the preparation of five-membered rings and these reactions have been successfully applied to many porphyrins and analogues. In some instances, nitrones have been chosen as 1,3-dipolar species when the goal is to prepare chlorin (monoadduct) or bacteriochlorin (bisadduct), however, unfortunately, these cycloadditions are reversible, suggesting that they are under thermodynamic control, which might explain the relatively low experimental yields obtained. Nevertheless, the isoxazolidine ring resulting from the cycloaddition of alkenes with nitrones have proven to be very useful as building blocks, and the cleavage of the isoxazolidine system allows access to a diversity of attractive compounds such as amino alcohols, amino acids, α,β-unsaturated aldehydes and others.

Herein, we present the synthesis of the isoxazolidine-fused chlorin 2, via 1,3-DC of meso-tetakis(pentafluorophenyl)porphyrin (1) with N-methyl nitrone, and a straightforward method for the preparation of a mono-annulated chlorin 4 having a 1-methyl-2,3-dihydro-1H-benzo[b]azepine ring system. Further treatment of 4 with NaH induces a second intramolecular nucleophilic aromatic substitution generating the bis-annulated chlorin 5 bearing an additional 2H-pyran ring. This represents the first example of a series of synthetic meso-tetraarylchlorins bearing a 2,3-dihydroazepine exocyclic ring, exhibiting unusual asymmetries and strong absorption bands in the region of 652-664 nm. Isoxazolidine-fused chlorin 2 was also cationized with methyl iodide providing chlorin 3. Considering that cationic porphyrin derivatives can efficiently photoinactivate microorganisms, cationic chlorins of type 3 may be useful for environmental or medicinal applications.

The synthesis of the isoxazolidine-fused chlorin 2 and further transformations are depicted in Scheme 1. Considering the previously described protocol, and after extensive screening of reaction conditions (solvents, reaction temperature vs time), the best result for the 1,3-DC of meso-tetakis(pentafluorophenyl)porphyrin 1 with N-methyl nitrene, generated in situ from N-methyl hydroxylamine hydrochloride and paraformaldehyde, was achieved in toluene at 60 °C, in the presence of potassium carbonate. The expected isoxazolidine-fused chlorin 2 was obtained in 71% yield. Crystallization of compound 2 from dichloromethane/methanol afforded green crystals with adequate quality for single crystal X-ray diffraction analysis, which confirmed unequivocally the structure of the isoxazolidine-fused chlorin (Figure 2). The solid-state structure was determined in the triclinic crystal system (space group P–1) with the asymmetric unit cell (asu) revealing only one unique molecule (detailed information about the crystallographic data collection and structure refinement are in supporting information). The X-ray structure shows that the isoxazolidine ring is nearly perpendicular to the porphyrin macrocycle, since the dihedral angle between the mean planes of the porphyrin unit and the isoxazolidine ring is 89.79(9)°. The crystalline packing of the 2 molecules is mainly directed by an extensive network of intermolecular interactions, namely weak hydrogen bonds of the type C–H···F and π–π stacking involving the C6F5 aromatic rings of adjacent molecules (SI: Figure S1).

Cationization of chlorin 2 with methyl iodide afforded the quaternary ammonium salt 3 in 17% yield. Surprisingly, a significant amount of porphyrin 1 was also isolated from the reaction mixture. In order to verify if the regeneration of 1 occurs via 2 or 3, a toluene solution of chlorin 2 (without addition of other reagents) was heated at 40 °C during 4 days. After this period, a moderate amount of 1 was obtained, which means that a retro-cycloaddition reaction takes place under these conditions.

The cleavage of the isoxazolidine N-O bond in chlorin 2 was firstly attempted using palladium-catalyzed hydrogenation conditions. Unfortunately, when the reaction was conducted under hydrogen atmosphere (4 bar), at room temperature, a mixture of bacteriochlorins, isobacteriochlorins and other products was obtained, presumably resulting from the hydrogenation of multiple double bonds in the porphyrin macrocycle. The microwave-assisted catalytic transfer hydrogenation described by the groups of Quinn and Kappe is known to be effective for the reduction of aromatic nitro groups to amino groups. Since this method allows a good control of the amount of generated hydrogen (proportional to the amount of cyclohexene added), and reduces significantly the time of the reaction, minimizing the occurrence of undesired secondary reactions, we anticipated that it could be suitable to promote the isoxazolidine ring-opening reaction in porphyrin derivatives.
Thus, this methodology was applied to the reaction of chlorin 2 with cyclohexene (70 equiv), in the presence of Pd/C, operating at 130 °C for 10 min. It was expected the formation of a chlorin bearing a secondary amine and an alcohol function but such compound was not obtained. Instead, a compound resulting from the opening of the isoxazolidine ring followed by a nucleophile aromatic substitution of the o-F atom of the adjacent aryl ring was achieved. Both NMR and tandem mass spectrometry (MS/MS) analysis support that the exocyclic ring resulted from the reaction of the pentafluorophenyl group with the generated secondary amine, and not with the hydroxy group. In fact, when comparing the $^1$H NMR spectra of compounds 2 and 4, the most surprising difference is the signal corresponding to the N-methyl group that appears as a doublet. This is due to a long-range coupling with the neighboring fluorine atom (the same effect also occurs in compounds 5 and 6, see infra). This is consistent with the nucleophilic substitution of the o-F atom by the amine group. Higher-energy collision dissociation (HCD-MS/MS) of the molecular ion at m/z = 1016.11 revealed the characteristic neutral losses of –HF (m/z = 996.1034) and –H$_2$O (m/z = 998.1008), the latter corresponding to reformation of the β-β double bond at the substituted pyrrole ring. Further evidence for the structure of chlorin 4 is provided by diagnostic low molecular weight fragments at m/z = 228.0424, m/z = 256.0374 and m/z = 283.0480 which bear the unusual 7-membered ring structure (SI: figure S30).

Treatment of a THF solution of chlorin 4 with NaH (15 equiv) at room temperature afforded chlorin 5 in 92% yield (Scheme 2), which resulted from a second nucleophilic aromatic substitution of the o-F atom of the adjacent aryl ring by the in situ generated alkoxide. In order to obtain the methoxy derivative 6 (Scheme 2), a similar reaction was carried out using a THF solution of chlorin 4, NaH (15 equiv), and a large excess of methyl iodide. However, a mixture of two compounds was obtained. These compounds were separated by preparative TLC and were identified as the bis-annulated chlorin 5 (67% yield) and the methoxy chlorin 6 (16% yield). Single-crystals of chlorin 5 were analyzed by X-ray diffraction methods, allowing to confirm the proposed structure for the bis-annulated chlorin (Figure 3). The crystal structure was determined in the triclinic space group P-1, with the asu showing two crystallographic independent molecules (details concerning the crystallographic data collection and structure refinement can be found in the supporting information). The structure of the molecule reveals a considerable level of asymmetry and deformation: the dihedral angles between the mean plane of the porphyrin center and the 2H-pyran ring and the seven member ring are 14.12° and 49.25°, respectively. The extended packing arrangement of the molecules is strengthened and stabilized by an extensive network of intermolecular interactions of the type C–H...F (SI: Figure S2).

The NMR analysis showed the increasing level of asymmetry and deformation going from the starting porphyrin 1 to chlorin 2 and the mono- and bis-annulated derivatives 4 and 5. This is mainly evidenced in the $^1$H NMR spectra both by changes in the multiplicity and the increasing value of the chemical shift corresponding to the NH protons, going from a singlet at -2.92 ppm in porphyrin 1, to the broad singlet displayed at -1.91 ppm in chlorin 2, two singlets at -1.81 and -1.68 ppm in 4 and also two singlets at -1.03 and -0.93 ppm in 5. The increasing level of asymmetry and the occurrence of mono- and bis-annulation is also evident in the $^{19}$F NMR spectra, where multiple signals corresponding to the resonance of total 20 F atoms in chlorin 2, falling to 19 F atoms in 4 and 18 F atoms in 5 are discernible (see Supporting Information).

The UV−vis spectra (Figure 4a) of the mono- and bis-annulated chlorins present the typical profile of a chlorin with a 9 nm red-shift observed for $\lambda_{max}$ of the Soret band for the mono-annulated chlorin 4 when compared to isoxazolidine-fused chlorin 2 and an additional 13 nm red-shift when regarding the bis-annulated chlorin 5. Also, the low energy Q-bands of 2, 4 and 5 are gradually red-shift, appearing at 646, 652 and 664 nm, respectively. We interpret the similarity of the chlorin and annulated chlorins as an indication that the conformational flexibility of the annulated systems is comparable to that of the non-annulated chlorin 2.

All fluorescence spectra show a chlorin-like intensity distribution of the two band emission spectra. When comparing the maximum emission wavelength, a red-shift of 9 nm is observed from 2 to 4 and an additional red-shift of 12 nm is observed when going from 4 to 5.

The quantum yields of synthesized chlorins are presented in Table 1. Chlorin 4 shows a fluorescence quantum yield similar to chlorin 2, indicating that mono-annulation, which leads to the formation of the more flexible seven-membered ring, does not influence the fluorescence properties of this chlorin. However, the bis-annulation, leading to the formation of the additional 2H-pyran ring, significantly decreases the fluorescence quantum yield of chlorin 5. This is possibly due to the increased deformation of the macrocycle, as revealed by the crystal structure of chlorin 5 (Figure 3). These results are consistent with those presented by Brückner.
with the presence of the biologically active azepine skeleton, red-shifted absorption and fluorescence spectra, which combined substitution takes place, yielding the bis-annulated chlorin intramolecular nucleophilic aromatic substitution. Upon treatment dihydro-1 spectrometers are part of the National NMR Network and were C/QUI/UI0062/2013 and PEst-C/EQB/LA0006/2011. The NMR COMPETE, projects NORTE-07-0162-FEDER-000048, NORTE -07- laboratory.
of other nitrones and further transformations are underway in our diagnosis and therapy of cancer. Investigations concerning the use might be of great importance for a potential application in the Table 1.

<table>
<thead>
<tr>
<th>Chlorin</th>
<th>Quantum Yield ((\varphi_F))</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.19</td>
</tr>
<tr>
<td>4</td>
<td>0.23</td>
</tr>
<tr>
<td>5</td>
<td>0.08</td>
</tr>
</tbody>
</table>

In summary, we have described an efficient synthetic approach to prepare the mono-annulated chlorin 4 carrying a 1-methyl-2,3-dihydro-1H-benzo[b]azepine ring, resulted from a reductive ring-opening reaction of the isoxazolidine fused-chlorin 2 followed by an intramolecular nucleophilic aromatic substitution. Upon treatment with NaH, a second intramolecular nucleophilic aromatic substitution takes place, yielding the bis-annulated chlorin 5 bearing an additional 2H-pyran ring. Both annulated chlorins display red-shifted absorption and fluorescence spectra, which combined with the presence of the biologically active azepine skeleton, might be of great importance for a potential application in the diagnosis and therapy of cancer. Investigations concerning the use of other nitrones and further transformations are underway in our laboratory.

This work was supported by Fundação para a Ciência e a Tecnologia (FCT, Portugal), European Union, QREN, FEDER and COMPETE, projects NORTE-07-0162-FEDER-000048, NORTE-07-0124-FEDER-000066/67, PTDC/QEQ-QOR/1273/2012, PEst-C/QUI/UI0062/2013 and PEst-C/EQB/IA0006/2011. The NMR spectrometers are part of the National NMR Network and were purchased in the framework of the National Programme for Scientific Re-equipment, contract REDE/1517/RMN/2005, with funds from POCI 2010 (FEDER) and FCT.

**Notes and references**


