

Synthesis of verdinochlorins: a new class of long-wavelength absorbing photosensitizers

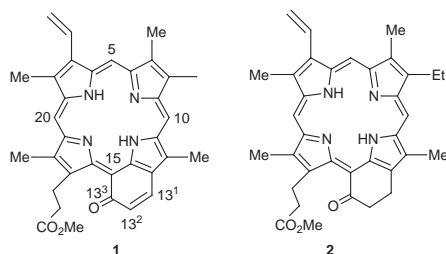
Andrei N. Kozyrev,^a James L. Alderfer,^b Thomas J. Dougherty^a and Ravindra K. Pandey^{*a}

^a Chemistry Section, Department of Radiation Biology, Division of Radiation Medicine, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

^b The Department of Biophysics, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

Reaction of 13²-oxopyropheophorbide **a** with CH₂N₂ produced three isomeric methoxyverdinochlorins *via* expansion of the cyclopentanedione ring E; these long-wavelength absorbing verdinochlorins represent the first example of verdins with a reduced pyrrolic ring system, and their structural assignments have been made on the basis of ¹H NMR studies; a mechanism of verdinochlorin formation is discussed.

Verdins are a special class of green porphyrins **1** with a fused



cyclohexenone ring system, which are obtained by the oxidation of the cyclohexanoneporphyrins or rhodins **2** under acidic reaction conditions.¹ Verdins conjugated exocyclic ring has a strong electron-withdrawing effect and causes a dramatic change in the electronic spectrum, with a prominent absorption near 690 nm.² Due to their intense green color, Fischer named this class of compounds as 'verdins' (from Latin *verdo* = green).¹ The chemistry of rhodins and verdins was further explored by Clezy, who prepared a variety of porphyrins with fused exocyclic rings.³ The utility of this class of compounds as photosensitizers for the treatment of cancer by photodynamic therapy (PDT) was demonstrated by Morgan *et al.*⁴

Recently, we have discovered a new and simple method for the preparation of 13²-oxopyropheophorbide **a** by LiOH-promoted allomerization of pyropheophorbide **a**.⁵ The oxopyropheophorbide **a** initially obtained as the carboxylic acid was readily converted into the corresponding methyl ester **3** by treating briefly with CH₂N₂. However, if a large excess of CH₂N₂ was used and the reaction mixture was left at room temperature for 4–6 h, a mixture of three main compounds was obtained. These were separated into individual components by preparative TLC. The most mobile orange band had a long-wavelength absorption at λ_{\max} 777 nm (29% yield), the second band was red in color (λ_{\max} 747 nm, yield: 26%) and the most polar compound was isolated as a green band with λ_{\max} 739 nm (yield: 15%) (Scheme 1). Significant bathochromic shifts in the electronic absorption spectra indicated the presence of a conjugated electron-withdrawing exocyclic ring system. Mass spectral analyses of all three products gave the same molecular ion at *m/z* 590. Thus, compared to chlorin **3** an increase in mass by 28 daltons is observed.

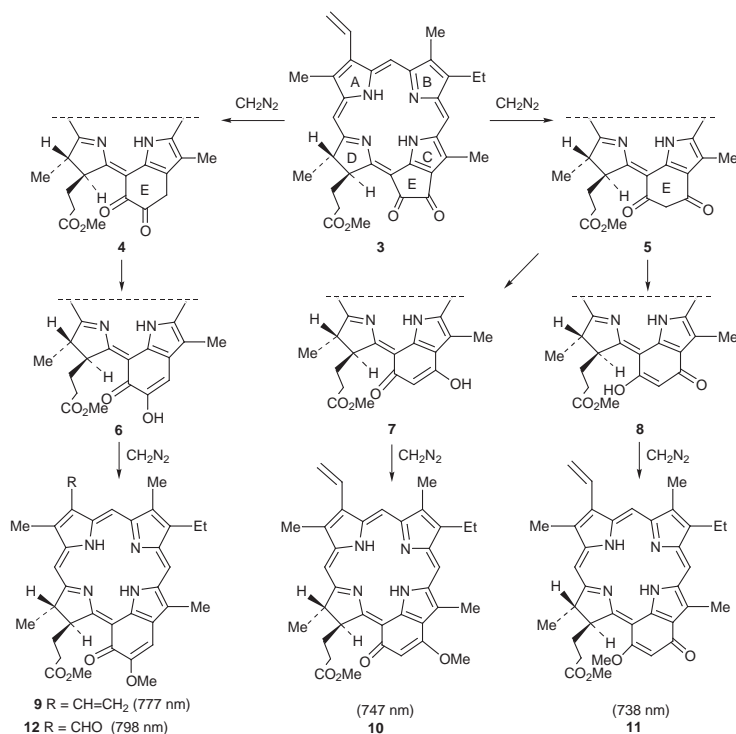
It has been shown that CH₂N₂ reacts with cyclic α -diketones,⁶ *e.g.* croconic acid, to produce trimethoxy-*p*-benzoquinone by an unusual ring expansion. Under these

reaction conditions, we anticipated the possibility of CH₂ insertion into the diketocyclopentane ring of chlorin **3**, which would yield the product(s) with fused cyclohexenone rings, named here as verdinochlorins (*i.e.* verdin with a reduced pyrrole ring).

The structural assignments of the predicted reaction products were confirmed by extensive ¹H NMR studies (Fig. 1). The ¹H NMR spectra revealed that, compared to starting diketochlorin **3**, all these compounds had an additional signal at δ 4.2, integrating for three protons, along with an additional singlet at δ 6.1 (for the chlorins having absorptions at 738 and 747 nm), and δ 6.85 (for the chlorin absorbing at λ_{\max} 777 nm) for the olefinic proton of the cyclohexenone ring, suggesting that all three products were structural isomers. The 2D ROESY NMR data further provided valuable information for the final structural identification. In the NMR spectrum, the orange band (λ_{\max} 777 nm) showed the most lowfield shifted signal of the proton at position 13¹ of the cyclohexenone ring, suggesting a closer proximity to the chlorin macrocycle. This was further confirmed by ROESY experiments which clearly showed the through-space interaction of the 13¹-olefinic proton with the resonances of the 13²-methoxy group and the 12-methyl substituent. Upfield shifted resonances for the 17-H proton (δ 5.1) indicated the presence of the neighboring keto group at the 13³-position.⁷ Based on these results, the structure for the fast moving band (λ_{\max} 777 nm) was assigned as 13²-methoxy-13³-oxoverdinochlorin **9**.

Both the red (λ_{\max} 747 nm) and green (λ_{\max} 739 nm) verdinochlorin isomers had resonances at δ 6.1 for the protons at the 13²-position. On the basis of the chemical shift of the 17-H, observed at δ 5.3 (similar to **9**), the structure of the red verdinochlorin was assigned as the 13¹-methoxy-13³-keto-isomer **10**. However, for the green isomer, the 17-H resonance was observed at δ 4.7, which suggested the structure to be that of 13³-methoxy-13¹-keto-chlorin **11**. In contrast to chlorins **9** and **10**, compound **11** is not a 'true' verdinochlorin, based on the position of the oxo group, and is named as 'isoverdinochlorin'.

The mechanism of the formation of the verdinochlorin isomers seems to be similar to that of the ring enlarged diketone obtained by CH₂N₂ treatment of croconic acid.⁸ The insertion of the CH₂ fragment into the cyclopentane dione ring (between the 13/13¹ and 13¹/13² carbon atoms) will produce diketocyclohexane intermediates **4** and **5** (Scheme 1). The enolization of the acidic hydrogens adjacent to the keto function will generate the isomeric hydroxyverdinochlorins **6–8**. The hydroxy functions can then react with a second molecule of CH₂N₂ to give the resulting methoxyverdinochlorins **9–11**. From the reaction mixture, we did not isolate the cyclohexanone analog in which the keto groups were present at positions 13² and 13³. This indicates that the reactivity of the 13²-keto group in diketochlorin **3** is diminished due to the steric hindrance caused by the substituents attached to the adjacent reduced pyrrolic ring. Thus, CH₂N₂ as nucleophile preferentially attacks the keto group present at the 13¹-position.



Scheme 1

Compared to most of the chlorins, all verdinochlorin isomers **9–11** showed a significant red shift of the long-wavelength absorption (Q_y -band) in their UV–VIS spectra, which makes them attractive candidates as photosensitizers for photodynamic therapy. It was interesting to observe that the position of the methoxy group in the cyclohexenone ring of verdinochlorins **9** and **10** plays an important role in the shift of the Q_y -band. Thus, compared to chlorin **3**, 13³-methoxyverdinochlorin **10** gave a red shift of 69 nm, while an even greater shift (99 nm) was observed for the 13²-methoxy isomer **9**. Both verdinochlorins **9** and **10** had Q_x -bands around 560 nm, while isoverdinochlorin **11** on the other hand showed Q_x -absorption at 600 nm. These absorptions are obviously responsible for their red and green color respectively.

In order to achieve a further bathochromic shift, the vinyl group in chlorin **9** (λ_{max} 777 nm) was replaced with a formyl substituent. The corresponding formyl analog **12** showed an unusually large red-shifted long-wavelength Q_y -band in the near-IR region (λ_{max} 798 nm). Owing to their long wavelength

absorptions in the red region, these compounds will have definite advantages over Photofrin® (a porphyrin derivative) for treating tumors which are deeply seated.

In conclusion, the new class of chlorins discussed here are the first examples of chlorins with a fused cyclohexenone rings. These compounds are obtained by the ring expansion of the fused cyclopetanedione ring attached to the chlorin system. This methodology has great potential in designing long-wavelength absorbing photosensitizers for PDT, and is currently being explored in our laboratory. The reactions discussed above might also be useful for the preparation of petroporphyrins with cyclohexane/phenyl ring systems. Biological *in vivo* PDT experiments with these compounds are currently in progress and will be published elsewhere.

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Notes and References

† E-mail: pdtctr@sc3101med.buffalo.edu

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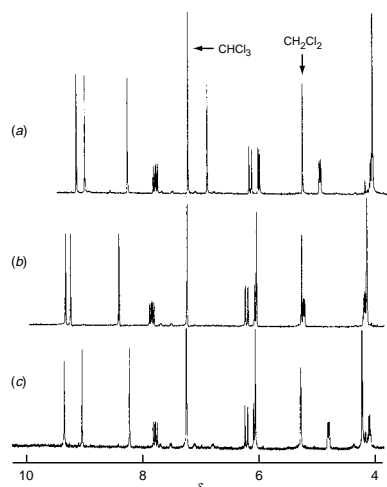


Fig. 1 ^1H NMR spectra (400 MHz) of (a) verdinochlorin **9**, (b) verdinochlorin **10** and (c) verdinochlorin **11**