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Cycloaddition reaction on 3-vinylemeraldins: formation of unexpected porphyrins with seven-membered exocyclic ring systems

Sashikumar Mettath,[a] Thomas J. Dougherty [a] and Ravindra K. Pandey [a,b,*]

[a] *Chemistry Section, Photodynamic Therapy Center, Roswell Park Cancer Institute, Buffalo, NY 14263, USA*

[b] *Department of Nuclear Medicine, Roswell Park Cancer Institute, Buffalo, NY 14263, USA*

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Abstract

Reaction of 3-vinylemeraldin with dimethyl acetylenedicarboxylate (DMAD) produced a benzoporphyrin derivative via [4+2] Diels–Alder adduct and an unexpected porphyrin containing a seven-membered ring system (yield 15%) fused at the 3- and 5-positions of the macrocycle. The benzoporphyrin derivatives containing *N*-alkylated fused imide ring system exhibit long wavelength absorptions near λ_{max} 725 nm. In preliminary in vivo screening some of these analogs were found to be more effective than the related protoporphyrin IX (PP-IX) based benzoporphyrin derivative (BPDMA).

Cycloaddition reactions are among the most powerful reactions available to the organic chemist. The ability to simultaneously form and break several bonds, with a wide variety of atomic substitution patterns, and often with a high degree of stereocontrol, has made cycloaddition reactions the subject of intense study.[1] In porphyrin chemistry, the [4+2] Diels–Alder reactions have been used by various investigators for converting porphyrins into chlorin systems. Callot et al.[2] were the first to show that protoporphyrin IX dimethyl ester can undergo cycloaddition reactions {[4+2] and [2+2]} with various dienophiles. Among such Diels–Alder adducts, a benzoporphyrin derivative (BPD)[3] obtained from protoporphyrin IX dimethyl ester and dimethyl acetylenedicarboxylate (DMAD) as its monocarboxylic acid form (BPDMA), when activated by 690 nm light, has shown great promise for treating age-related macular degeneration (AMD), a major cause of blindness which currently has no cure. In an initial study, 40 patients were treated with BPDMA. In all the patients eyesight deterioration was halted and there were no short-term adverse effects. BPDMA has also been used for the treatment of cancer by photodynamic therapy (PDT).[4] However, due to its rapid clearance, it was found to be effective only if the tumors were treated with light at 3 h post injection of the drug.

* Corresponding author.

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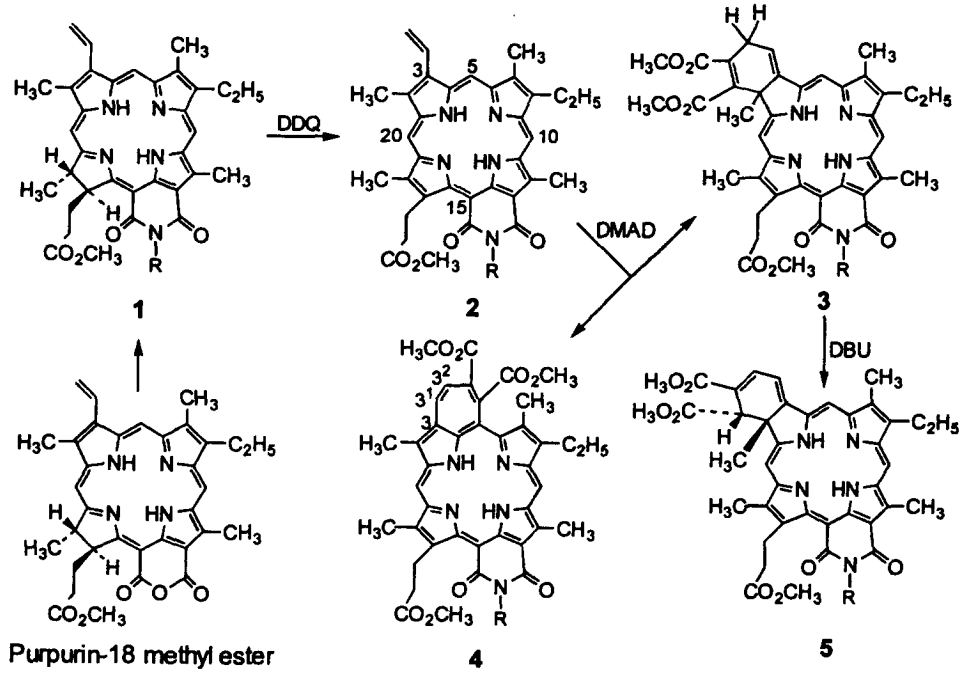
One of the main synthetic problems associated with PP-IX based benzoporphyrin derivatives is to isolate the most effective analog (ring-A reduced, mono-carboxylic acid) from the complex reaction mixture.[3] In order to solve this problem, we[5] and others[6] have previously reported the preparation of various BPD analogs from phylloerythrin and rhodoporphyrin XV as methyl esters and the corresponding aspartyl amides. Among these compounds, the benzoporphyrin derivative (*cis*-isomer) obtained from rhodoporphyrin XV di-*tert*-butyl aspartate was found to show PDT efficacy similar to BPDMA.[7] However, replacement of the vinyl group (ring B) of the PP-IX based BPD with a (1-hexyloxyethyl) side chain enhanced the PDT efficacy.[8] These results along with our recently published work on certain chlorophyll-a related compounds[9] suggest that lipophilic characteristics of the molecules certainly play an important role in tumor localizing ability.

In our attempts to further explore the effect of various substituents on PDT efficacy, our aim was to synthesize and evaluate the BPD analogs of porphyrins containing the fused imide ring system **2**. In this molecule, the lipophilicity can be easily altered by introducing various *N*-substituents at the fused imide ring, and thus offers a unique ability to understand the structure–activity relationship in a particular series of compounds (Scheme 1).



Scheme 1. (a) R=methyl; (b) R=*n*-hexyl; (c) R=*n*-dodecyl

For our studies, purpurin-18 methyl ester, obtained from methylpheophorbide-a was converted into the related *N*-alkyl analogs **1(a,b,c)** by following the methodology developed in our laboratory.[10] Brief treatment of *N*-alkylpurpurinimides, e.g. **1b** with methanolic DDQ, produced emeraldin **2b**[10] in 60% yield with long wavelength absorption at λ_{max} 663 nm (ε: 13 500). Reaction of **2b**, containing a vinyl group at position-3 (ring-A) with dimethyl acetylenedicarboxylate in refluxing toluene for 3 days, gave a mixture of mainly two compounds. The slower moving band on silica gel, a [4+2] adduct **3b** [*m/z* 803 (M+1)] was obtained as a major component which upon DBU treatment gave the expected benzoporphyrin derivative (*cis*-isomer) **5b** in 40% yield. Replacing DBU with triethylamine produced a complex reaction mixture. The structure of the Diels–Alder adduct **3b** and the corresponding

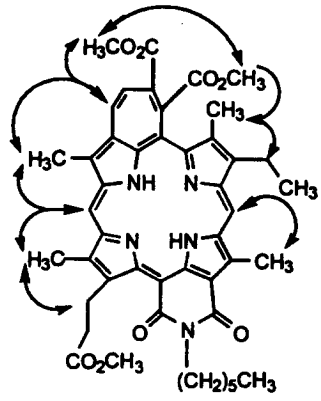


Figure 1. 2D NOE connectivities

base-promoted benzoporphyrin derivative **5b** was confirmed by NMR and mass spectrometry analyses (including 2D ROESY NMR) studies (Fig. 1). Compared to the resonances for various protons in starting porphyrin **1b**, the NMR spectrum of **3b** showed significant upfield shifts in the resonances of the two *meso* and one ring-methyl protons, which appeared at δ 9.23, 9.13 and 2.12 ppm, respectively. These results suggested the formation of a reduced pyrrole unit with an exocyclic ring system. The exocyclic ring protons at 3^1- and 3^2-positions were observed at δ 7.33 (dd, 1H), 4.01 and 3.95 ppm (each 1H, non-equivalent protons), respectively. In the DBU rearranged product **5b**, the protons associated with the exocyclic ring appeared at δ 7.82 (d, 1H, *J*=6 Hz,), 7.43 (d, 1H, *J*=5.5 Hz) and 5.04 ppm (s, 1H). The other *N*-alkyl analogs **4** and **5** (**a**,**c**) were prepared by following the same methodology, and were obtained in similar yields.

On the basis of the mass spectral analyses [*m/z* 801 (M+1)], the structure of the minor product was initially assigned as **4**. The presence of only two *meso* protons and the absence of vinyl resonances suggested the involvement of the vinyl and the adjacent *meso* proton in the formation of an additional ring system. The 2D ROESY NMR data confirmed the predicted structure. In the NMR spectrum, the *meso* proton observed at δ 9.97 ppm (position-20) showed a strong interaction with resonances at δ 3.75 and 3.52 ppm and were assigned to 2- and 18-methyl protons, respectively. The methyl protons observed at δ 3.75 ppm produced a strong interaction with an aromatic proton (3^1) observed at 9.00 ppm (d, 1H, *J*=9.7 Hz), which in turn had interaction with an adjacent aromatic proton (3^2) appearing at δ 7.82 ppm (d, 1H, *J*=9.6 Hz). On the basis of the interactions of the 3^2 protons, and the following assignments for the methyl protons observed at δ 4.05 and 3.15 ppm, these resonances were confirmed for the methoxycarbonyl groups present at 3^3- and 3^4-positions of the newly formed seven-membered ring system. Following a similar approach, the resonances for the other protons were also assigned.

The mechanism for the formation of the fused seven-membered ring in compound **4** is not clear. Despite extensive studies of Diels–Alder reactions in porphyrin systems by us and others, this is the first example which illustrates the formation of such a novel system in 12–15% yield. As shown in the electronic absorption spectra of the emeraldin and the other reaction products (measured in dichloromethane), the long wavelength absorptions for the seven-membered emeraldin **4** and the benzoporphyrin derivative **5** were observed at λ_{max} 708 nm (ε: 11 532) and λ_{max} 723 nm (ε: 13 566), respectively (Fig. 2). Thus, compared to the protoporphyrin IX based BPD analog (BPDMA, λ_{max} 690 nm), the BPD derivative **5b**, containing a fused imide ring system, exhibited a red-shift of about 43 nm. Due to its substantially longer wavelength absorption in the red portion of the spectrum, this class of BPD analogs might have an advantage in treating tumors which are deeply seated. The presence of the additional six- and seven-

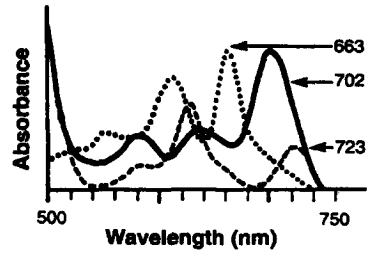


Figure 2. Absorption spectra of **2** (····), **4** (—) and **5** (- - -)

membered exocyclic ring in **4** and **5** also produced a significant red shift into the corresponding Soret bands, appearing at 452 nm.

In our attempts to understand more about the effect of the lipophilic characteristic of the molecule in in vivo PDT efficacy, and also to establish the structure–activity relationships (SAR) in a particular series of compounds, two related *N*-alkyl substituted BPD analogs (*N*-methyl **5a** and *N*-dodecyl **5c**) with one (least hydrophobic) and 12 carbon units (most hydrophobic) were initially synthesized. In preliminary in vivo testing, the corresponding *N*-hexyl (**5b**) and *N*-dodecyl (**5c**) analogs were found to be quite effective (six animals per group were injected intravenously with 0.5 μmol/kg of photosensitizer and subjected to 728 nm light (in vivo absorption) at 75 mW/cm^2 in a total dose of 135 J/cm^2 approximately 24 h post-injection of the drug). Under similar treatment conditions the BPDMA, obtained from protoporphyrin IX dimethyl ester (treated with light at 690 nm, the long wavelength in vivo absorption of BPDMA), did not produce any photosensitizing efficacy. The detailed biological studies with these and other related *N*-alkyl analogs with variable lipophilicity at different doses and time intervals are currently in progress, and these results will be published in our full paper.

The Diels–Alder approach discussed here also provides a simple approach for the preparation of porphyrins featuring a tetramethine bridge joining the 3- and 5-positions of the macrocycle, which are otherwise obtained by multistep synthesis.[11] This class of compounds has also been used by Gust and co-workers[11] to produce a porphyrin macrocycle linked through a rigid, bicyclic bridge to construct the multicomponent models for photosynthesis and other biological processes.

Acknowledgements

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