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EFFICIENT SYNTHESES OF NEW CLASSES OF REGIOCHEMICALLY PURE BENZOPORPHYRIN DERIVATIVES

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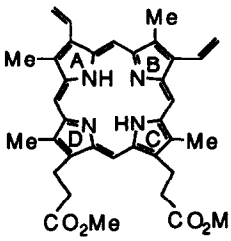
Abstract: Starting from rhodoporphyrin-XV dimethyl ester **4** and phylloerythrin methyl ester **5** new classes of isomerically pure benzoporphyrin analogues **7, 8, 11-17** were synthesized in high yields. These compounds have strong long wavelength absorptions in the red region and demonstrate activity against a transplanted mouse tumor.

In recent years a variety of photosensitizers absorbing at wavelengths between 660 and 800 nm, to obtain maximum tissue penetration, have been reported.[1] Among such photosensitizers, the so-called benzoporphyrin derivatives (BPDs) have generated interest due to their low skin phototoxicity compared with Photofrin, the industry standard. As a result, "BPD" (as a mixture of two monomethyl esters) is one of the long wavelength photosensitizers which are currently in Phase-I clinical trials. For synthesis of the active component of BPD[2] (with ring "A" modified), protoporphyrin IX dimethylester **1** is reacted with dimethyl acetylenedicarboxylate (DMAD) and subsequently rearranged with base (DBU). The ring A and ring B Diels-Alder adducts are then separated by column chromatography. The ring "A" adduct (the more active component) is then partially hydrolyzed to afford the BPD mixture (isomers **2** and **3**) which are used as a mixture for biological studies.[3] From the limited biological data reported so far, it seems that there are two main structural requirements for a biologically active BPD derivative: (a) only ring "A" of the porphyrin nucleus should be modified, (b) it should have only one propionic acid side chain.



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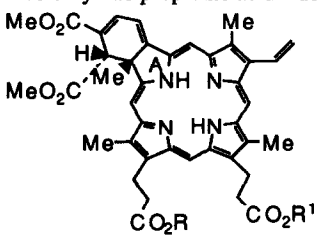
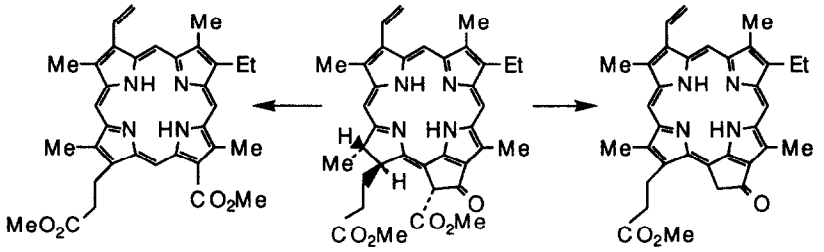


2 R = H, R^1 = Me
3 R = Me, R^1 = H

We have recently reported new synthetic approaches for the preparation of BPDs.[4,5] In one such route,[5] 4-acetyl-2-vinyldeuteroporphyrin-IX dimethyl ester was used as a starting material, obtained after dehydrating the corresponding (1-hydroxyethyl) derivative.[6] Though this method has certain advantages over the earlier methodology, it still requires chromaotgraphic separation of two isomers [2-acetyl-4-(1-hydroxyethyl)- and 4-acetyl-2-(1-hydroxyethyl)-deuteroporphyrin-IX dimethyl ester].[7] They can, however, be readily obtained in gram quantities. We have also reported a series of photosensitizers prepared from the chlorophyll-a derivatives, pheophorbide-a, pyropheophorbide-a and chlorin-e_6.[8,9] Preliminary *in vivo* results led us to conclude that the five-membered isocyclic ring (ring E) plays an important role in photosensitizing ability of

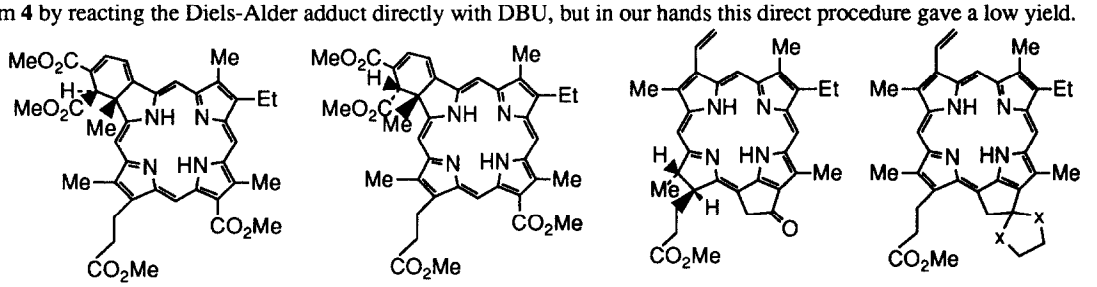
these compounds.[8,9] Furthermore, compounds derived from the pyropheophorbide series were found to be more active than the corresponding pheophorbide analogues.

In order to compare the structure activity relationships among chlorins, pheophorbides and BPDs and also to understand the basic requirements for an effective long wavelength photosensitizer, we have prepared a series of novel BPDs. Two series of photosensitizers related to BPD were synthesized by using rhodoporphyrin-XV dimethyl ester **4** and phylloerythrin methyl ester **5** as starting materials. These are ideal substrates to probe structure/activity relationships in the BPD series because both systems have a vinyl group at position 2 (i.e. in ring "A", and thus will produce only ring "A" chlorin after the Diels-Alder reaction), and have only one propionic ester side chain, which can be hydrolyzed to the corresponding monocarboxylic acid at the final step of the synthesis.

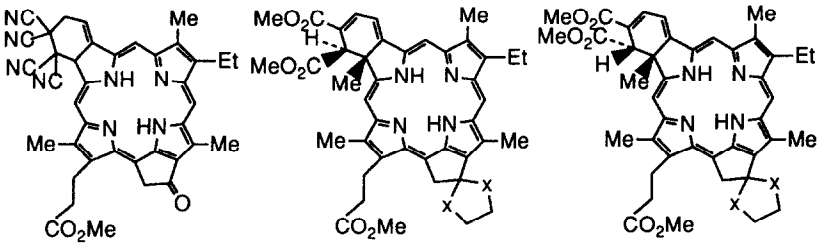


4 **6** **5**

Thus, for the synthesis of rhodoporphyrin-XV dimethyl ester **4** and phylloerythrin methyl ester **5**, methyl pheophorbide-a **6** extracted from *Spirulina pacifica* alga[8] was used. For the preparation of BPDs **7** and **8**, reaction of rhodoporphyrin-XV dimethyl ester[10] **4** with dimethyl acetylene dicarboxylate (DMAD) followed by rearrangement of the intermediate with triethylamine gave the BPD **7** (trans isomer λmax 662 nm).[5] Further treatment of **7** with DBU produced the cis-isomer **8** in which the long wavelength absorption maximum shifted to 668 nm. The overall yield of the desired isomer was 23%. The cis isomer **8** can be obtained directly from **4** by reacting the Diels-Alder adduct directly with DBU, but in our hands this direct procedure gave a low yield.



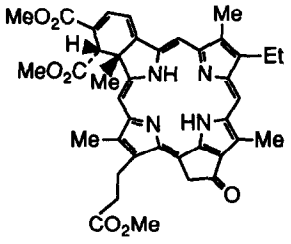
7 **8** **9** **10**



11 **12** **13**

For the preparation of the BPDs bearing an isocyclic ring, methyl pyropheophorbide-a **9** was used (obtained in >95% yield from methyl pheophorbide-a **6** by refluxing with collidine).[10] The synthesis of phylloerythrin required the preparation of the 9-ketal **10** (X = O) of methyl pyropheophorbide-a before DDQ oxidation of the chlorin to porphyrin.[11] The ketal was easily cleaved by stirring with acidic aqueous acetone to give **5**. Diels-Alder reaction of **5** with tetracyanoethylene in refluxing chloroform

under nitrogen gave the desired chlorin **11** in 60% yield. No reaction was observed when a less reactive dienophile such as DMAD was used under similar conditions or even at elevated temperatures. However, reaction of ketal **10** (X = O) under similar conditions to those discussed for benzochlorin **7** produced the desired analogues **12** and **13**. Thus, it appears that the remote electronegative keto group attached to the isocyclic ring inhibits the Diels-Alder reaction. During the purification of the BPD we noticed that the ketal group tended to cleave during silica column chromatography, thus making the purification extremely difficult. In order to avoid this problem, the keto group in methyl pyropheophorbide-a was protected as the more stable thioketal **10** (X = S), and the desired BPD **14** was isolated in an overall yield of 40%. Like benzochlorins **7** and **8**, the cis-isomer **14** has a longer wavelength absorption (675 nm) than the corresponding trans-isomer **15** (663 nm).



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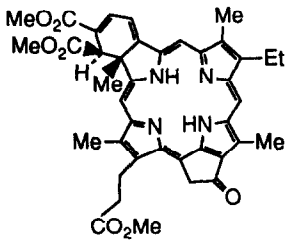

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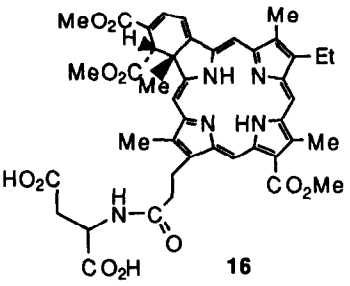
For certain photosensitizers, aspartic acid derivatives have been reported to show better photosensitizing efficacy than the corresponding methyl esters or carboxylic acids.[12] In order to compare the biological activity of such derivatized BPDs, the BPD **8** was first hydrolysed to the corresponding carboxylic acid (25% HCl/THF) and then converted to the aspartyl amide derivative **16** by following standard methodology. In the case of the aspartyl derivative **17**, the ketal or thioketal derivative methyl ester **10** was first hydrolyzed to carboxylic acid (aqueous LiOH/THF/methanol), and then converted to aspartyl derivative in quantitative yield before the Diels-Alder reaction and base-promoted rearrangement.



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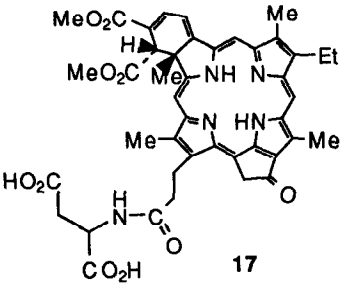

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Our new methodology has definite synthetic advantages over the reported method;[2,5] our new photosensitizers can be obtained in modest yield as a single isomer without tedious purification. The newly synthesized BPDs were evaluated for *in vivo* biological activity by following the standard procedures[13] used in our laboratories. In preliminary screening some of these compounds [**7**, **12**, **13** (X = O, S)] were found to be at least as active as BPDs prepared from protoporphyrin-IX dimethyl ester.[3] Further biological studies with photosensitizers **7**-**17** at different doses and time intervals are in progress, and the results will be reported in a full paper.

All the new compounds were characterized by proton NMR, spectrophotometry, elemental analysis, and/or high resolution mass spectroscopy. Detailed biological studies are in progress and will be reported elsewhere.

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11. Methyl pyropheophorbide a (1.45 g) was dissolved in CH_2Cl_2 (200 ml) and ethylene glycol (for ketal, **10**, X = O) or 1,2-ethanedithiol (for thioketal, **10**, X = S) (1.5 ml) and TMSiCl (1.5 ml) along with water (10 drops) were added; the mixture was stirred for 24 h. After a standard organic workup, the crude mixture was heated with isopropyl alcohol to leach out excess of ethylene glycol or 1,2-ethanedithiol. The solid so obtained was filtered, petroleum ether was added, and the mixture was refluxed and then cooled. The solid product was dissolved in CH_2Cl_2 and then passed through a short column of neutral alumina (Brockmann Grade III) using 0.25% methanol and 2% THF in CH_2Cl_2 as eluent. The solvent was evaporated and the residue was crystallized from CH_2Cl_2/petroleum ether to give 70-80% yields of **10**, X = O or S.

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13. Non-palpable tumors, 4-6 mm in diameter, were exposed to 75 mW/cm^2 light for 30 min to deliver 135 J/cm^2 from a tunable dye laser tuned to the maximum of the red absorption peak.