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# NEW SYNTHESES OF BENZOPORPHYRIN DERIVATIVES AND ANALOGUES FOR USE IN PHOTODYNAMIC THERAPY

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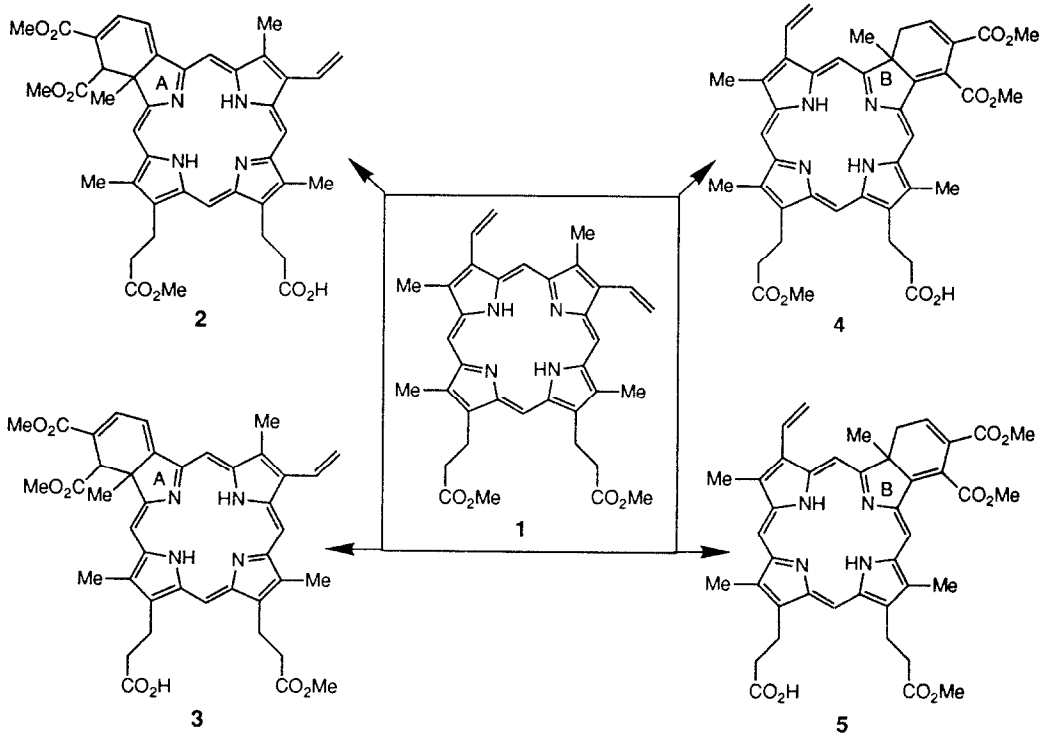
**Abstract.** An efficient route for the synthesis of isomerically pure benzoporphyrin derivative (BPD) is presented. To understand more about structure/activity relationships in photodynamic therapy, a series of BPD derivatives, including dimers linked with carbon-carbon bonds were also prepared. The structure of the most effective (ring A) BPD isomer (**14**) was confirmed by a single crystal X-ray study.

Among long-wavelength "second generation" photosensitizers for photodynamic therapy (PDT) benzoporphyrin derivatives (BPD) has attracted attention due to its low skin phototoxicity compared to Photofrin®.[1] BPD itself has recently entered clinical trials. BPD is prepared by first reacting protoporphyrin-IX dimethyl ester **1** with dimethyl acetylenedicarboxylate (DMAD). The Diels-Alder adduct thus obtained is rearranged in base to give the conjugated "benzoporphyrin system".[2] Though both ring A and ring B Diels-Alder adducts are equally useful, Levy and coworkers[1] have shown that the monomethyl esters (**2** and **3**) of the ring A protoporphyrin-IX derivative are most effective as PDT sensitizers. In principle, there are several synthetic problems in efficiently obtaining the pure isomers **2** or **3**. Firstly, treatment of protoporphyrin-IX dimethyl ester **1** with DMAD yields two Diels-Alder adducts (ring A and ring B). Then, partial hydrolysis of each of these two isomers must statistically afford two isomers, depending upon which ester of the 6,7-pair is hydrolyzed (Scheme 1). Protoporphyrin-IX dimethyl ester therefore affords no less than four isomeric monomethyl ester benzoporphyrin derivatives **2-5**.

In our approach, the well characterized isomerically pure 4-acetyl-2-vinyl- **6** and 2-acetyl-4-vinyl-deuteroporphyrin-IX dimethyl ester **7** were used as starting materials. These isomers were prepared by dehydration of the corresponding (1-hydroxyethyl) derivatives **8** and **9**, which in turn were prepared as a mixture of isomers, either by partial oxidation of hematoporphyrin-IX dimethyl ester **10**, using 4-methylmorpholine N-oxide and tetra-propylammonium per-ruthenate, or by partial reduction of 2,4-diacetyldeuteroporphyrin-IX dimethyl ester **11** with sodium borohydride.[3] The mixture was then readily separated in gram quantities using preparative scale high performance liquid chromatography to give individual isomers **8** and **9**.[4] Treatment of 4-acetyl-2-vinyl-deuteroporphyrin dimethyl ester **6** with DMAD in refluxing toluene for 7 to 8 days (monitored by spectrophotometry and TLC) gave the intermediate adduct **12** (Scheme 2). Treatment of **12** with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave the rearranged product **13a** in only 20% yield along with decomposition products. However, when the intermediate was first treated with triethylamine (TEA), followed by DBU the desired rearranged product was isolated in 40% yield. Compound **13b** obtained with TEA has a long wavelength absorption at 690 nm; further treatment with DBU produced a red shift to 696 nm. The 4-acetyl derivative **13a** was then converted into the benzoporphyrin derivative dimethyl ester **14** by reducing the acetyl group to give the (1-hydroxyethyl)-benzoporphyrin **15** followed by acid catalyzed dehydration in refluxing o-dichlorobenzene in 70% overall yield. The structure of **14** was confirmed by a single crystal X-ray study,

which showed (Figure 1) the angular methyl group and adjacent C-2' methoxycarbonyl group to be *trans* to each other.




**Scheme 1:** Possible BPD isomers (**2-5**) obtainable from mono-Diels-Alder reaction and mono-ester hydrolysis of protoporphyrin-IX dimethyl ester (**1**)

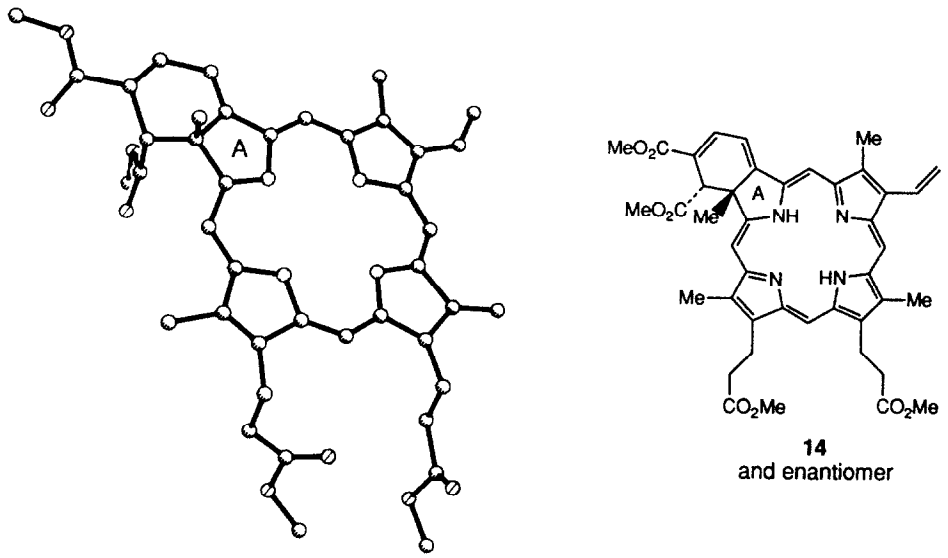
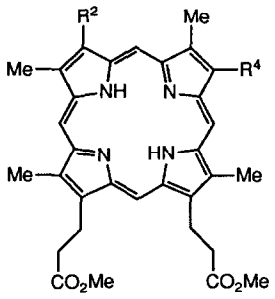
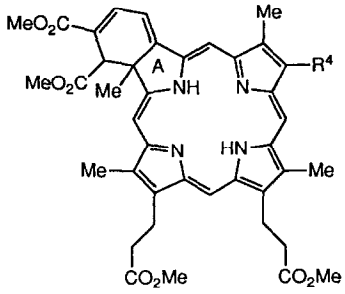


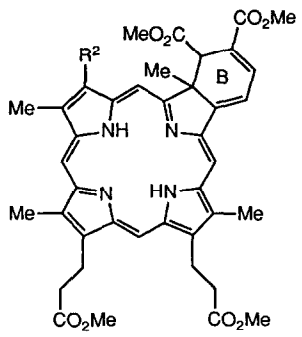

**Figure 1:** X-ray crystal structure of ring A Diels-Alder adduct **14**



**6** $R^2 = CH{=}CH_2$; $R^4 = CO.Me$
**7** $R^2 = CO.Me$; $R^4 = CH{=}CH_2$
**8** $R^2 = CH(OH)Me$; $R^4 = CO.Me$
**9** $R^2 = CO.Me$; $R^4 = CH(OH)Me$
**10** $R^2 = R^4 = CH(OH)Me$
**11** $R^2 = R^4 = CO.Me$

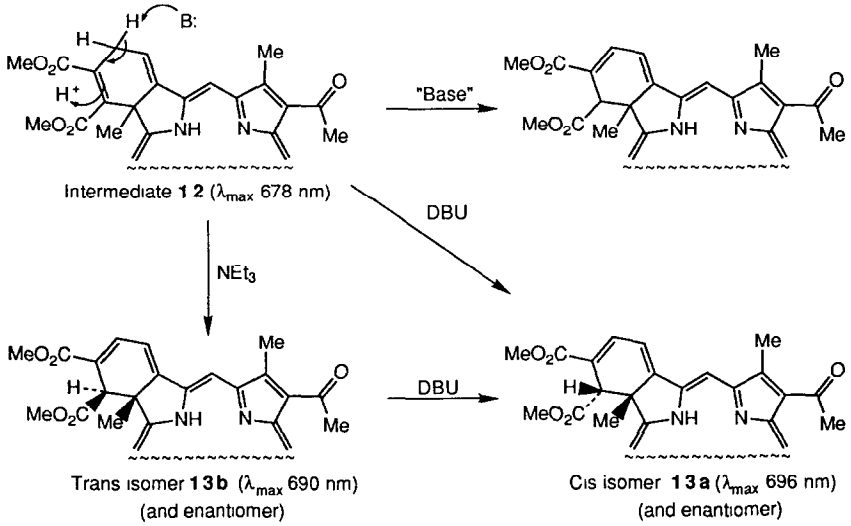


**13** $R^4 = CO.Me$
**14** $R^4 = CH{=}CH_2$
**15** $R^4 = CH(OH)CH_3$
**16** $R^4 = CHO$
**18** $R^4 = CH(OHexyl)Me$



**17** $R^2 = CHO$
**19** $R^2 = CH(OHexyl)Me$
**20** $R^2 = CO.Me$
**21** $R^2 = CH(OH)Me$
**22** $R^2 = CH{=}CH_2$

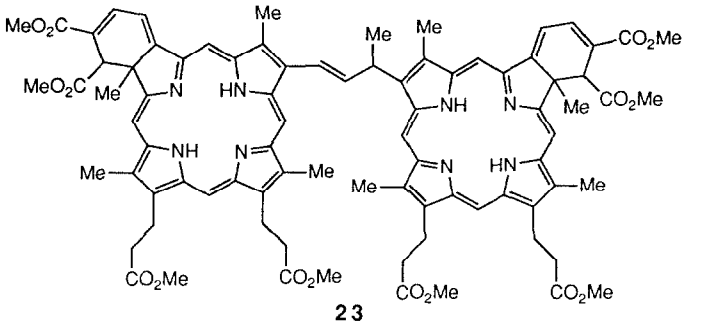
Dolphin et al.[2b] have reported a similar observation with the Diels-Alder adducts obtained from protoporphyrin-IX dimethyl ester. Starting from 2-acetyl-4-vinyldeuteroporphyrin-IX dimethyl ester **7**, the benzoporphyrin derivative **22** was prepared by following the same methodology and was isolated in an overall yield of 30%.



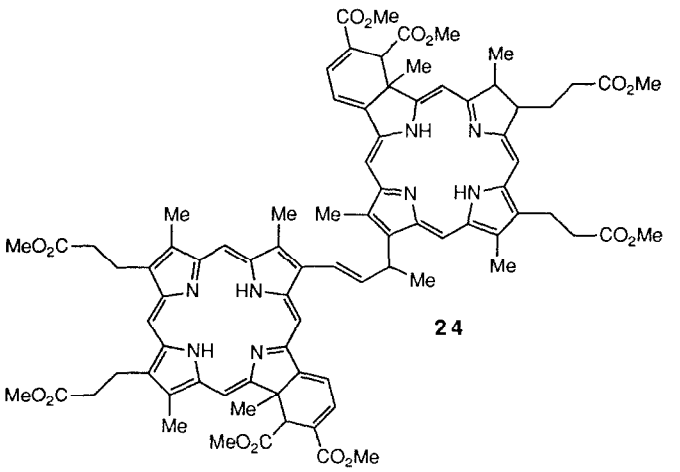

**Scheme 2**: Reactions of Diels-Alder adducts with $NEt_3$ and/or DBU

In the pheophorbide series we observed[5] that replacement of the 2-vinyl with formyl afforded a significant increase in photosensitizing activity. In order to permit further investigation of the therapeutic effect of the formyl group, 4-formyl-and 2-formyl-benzoporphyrin derivatives **16** and **17** were prepared by reacting the corresponding vinyl analogue with osmium tetraoxide and sodium periodate.[6] These formyl derivatives absorb strongly at $\lambda_{max}$ 696 nm. The isomeric structures of both compounds were confirmed by nuclear Overhauser enhancement experiments.

In a series of alkyl ether analogues of porphyrin,[7] as well as chlorins and pheophorbides[5,8] it has been shown that tumorcidal activity of the sensitizer increases with increasing the length of carbon units in the ether moiety; the hexyl ether derivatives, in particular, showed excellent tumorcidal activity. To ascertain the effect of the hexyl ether side chain on photosensitizing efficacy in the benzoporphyrin series, the BPD derivative **15** was first treated with HBr/acetic acid. The (1-bromoethyl) derivative was not isolated but was redissolved in dry n-hexanol. The desired product hexyl ether **18** was isolated in 52% yield as a mixture of diastereomers. The BPD derivative **19** was prepared from **20** (via **21**) under similar reaction conditions. In the $^1$HNMR spectrum of **18** (A ring modified), the C*H* protons (distant from the chiral angular methyl group in ring A) were observed as a multiplet at 6.02 ppm, integrating for one proton. However, in the other isomer **19** (B ring modified) the C*H*(O-Hexyl)$CH_3$ proton, which is closer to the angular methyl group, was observed as two separate quartets at 6.02 and 6.15 ppm, each integrating for one-half of a proton.



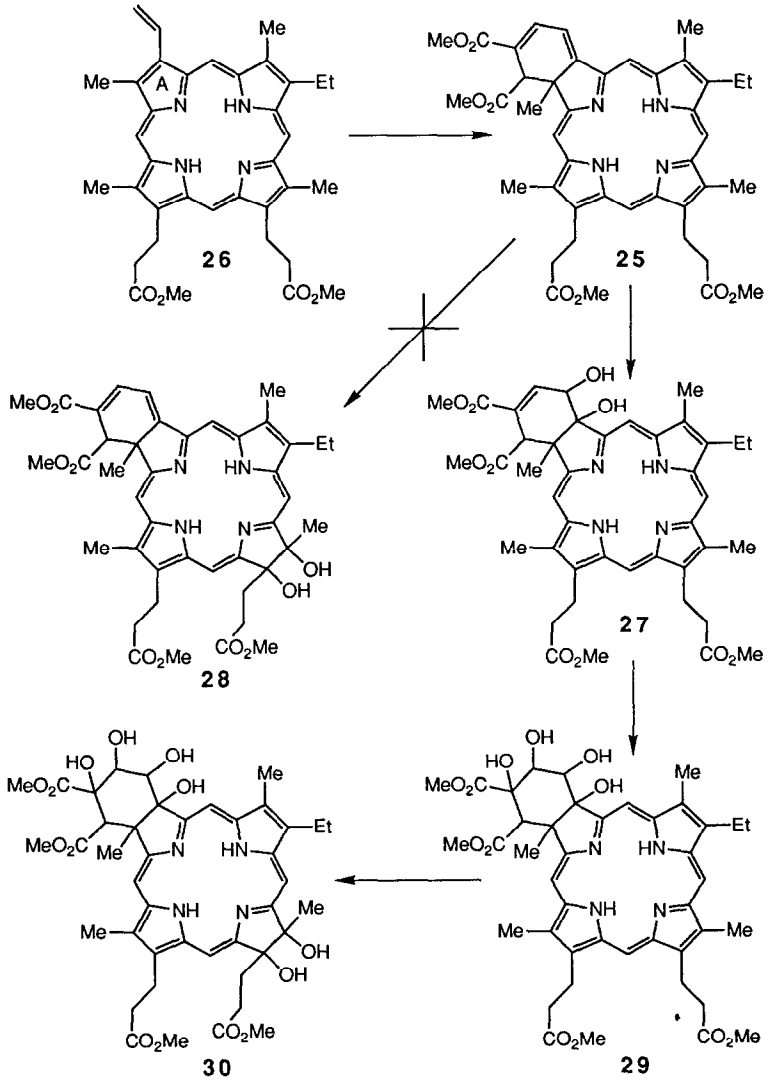

**23**




**24**

In the porphyrin series we and others have observed that some monomers which are biologically inactive in PDT demonstrate a significant increase in biological activity when they are converted into bis-porphyrins linked with carbon-carbon bridges.[9,10] Such a phenomenon is also possible in the benzoporphyrin series, which might thereby enhance the known tumorcidal activity of BPD. Thus, for the preparation of dimer **23**, benzoporphyrin derivative **15** was dissolved in dry dichloromethane and then treated with triflic acid[11] and was isolated in 58% yield. Under similar reaction conditions benzoporphyrin derivative **20** (via **21**) gave bis-benzoporphyrin **24** in 44% yield.

Chang and coworkers[12] have shown that the osmium tetraoxide reaction with porphyrins to give vic-diol-porphyrins can be directed to give vic-bacteriochlorins if the substrates in the reaction are metal-free vic-diol-porphyrins. Using this methodology, we have synthesized a series of bacteriochlorin analogues of natural chlorophyll derivatives.[13] However, when benzoporphyrin derivative **25** (nominally a "chlorin"), obtained in 40% yield from **26**, was reacted with osmium tetraoxide, the diol **27**, in which the reaction had occurred at the double bond in the carbocyclic ring, was obtained as a major product ($\lambda_{max}$ 652 nm). Formation of bacteriochlorin **28** was not observed even in trace quantities. Treatment of **25** with a large excess of osmium tetraoxide produced mainly tetraol **29** ($\lambda_{max}$ 642 nm), wherein the reaction had occurred to both of the double bonds in the carbocyclic ring, along with bacteriochlorin **30** ($\lambda_{max}$ 708 nm) and its dehydration products as minor components.



The *in vivo* biological studies with the compounds described above are in progress and will be reported elsewhere. All the new compounds were characterized by proton NMR, elemental analysis and/or high resolution mass spectroscopy.

**Acknowledgments**

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4. HPLC conditions: (*Preparative*) Waters Associates Prep LC3000 system attached to Waters 1000 PrepPak module with PrepPak-500 silica gel cartridge; 2.5% THF in $CH_2Cl_2$; 50.0 mL/min; Waters 484 tunable absorbance detector set at 405 nm. (*Analytical*) Waters Associates 510 pump, 600E solvent delivery system; Waters μPorasil 10μm stainless steel column (30 cm x 3.9 mm i.d.); 3% THF/$CH_2Cl_2$; 3.5 mL/min; Waters 490E programmable multiwavelength detector set at 405 nm; Retention times: **8**, 16 min; **9**, 19 min.

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