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REGIOSELECTIVE SYNTHESES AND SOME *IN VIVO* PROPERTIES OF "BENZOCHLORIN" ANALOGUES PREPARED FROM METHYL 9-DEOXYMESOPYROPHEOPHORBIDE

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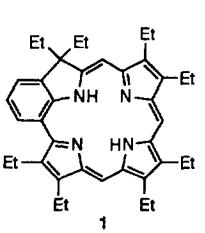
Abstract: Starting from methyl 9-deoxymesopyropheophorbide-a **5**, regioselective synthesis of a series of benzochlorins was achieved. These compounds have long wavelength absorptions in the range of 710-756 nm. The *in vivo* spectroscopic properties as well as the uptake and clearance characteristics of some of these compounds (e.g. **13**) were determined by use of *in vivo* reflection spectroscopy.

Benzochlorins (e.g. **1**) were first reported in the literature in 1978.[1] These compounds were subsequently studied in depth by Morgan et al.,[2] Smith et al.[3] and Gunter et al.[4] and were usually prepared either from octaethylporphyrin (OEP) **2** or 5,15-diphenyletioporphyrin. Due to their strong long-wavelength absorptions, these compounds were also evaluated for the treatment of cancer using photodynamic therapy (PDT). The benzochlorin **1**, obtained from OEP, has been reported to be an effective photosensitizer.[5] As with Photofrin®[6] (a mixture of porphyrin ologomers extensively used in PDT), the mechanism of action for the destruction of tumors by benzochlorin **1** is believed to be due to the sensitized production of singlet oxygen. Recently, Skalkos et al.[7] prepared a series of metal complexes of the iminium salt of benzochlorin **1**. Among these derivatives, copper(II) benzochlorin **3** was reported to be an excellent PDT sensitizer. Due to the paramagnetic metal ion in sensitizer **3**, it is not expected to form singlet oxygen, and thus tumor destruction using it might be due to the production of free radicals after irradiation with laser light (a so-called Type-I oxygen dependent mechanism). The Type-I mechanism is based on the assumption that the short-lived electronically excited state of the sensitizer (singlet/triplet) reacts directly either with substrate or solvent by electron transfer to form radical ions.[7]



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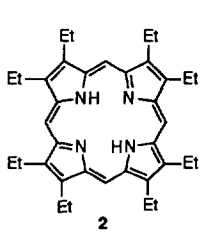

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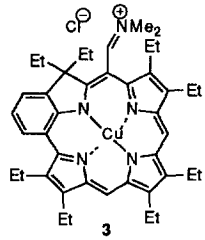

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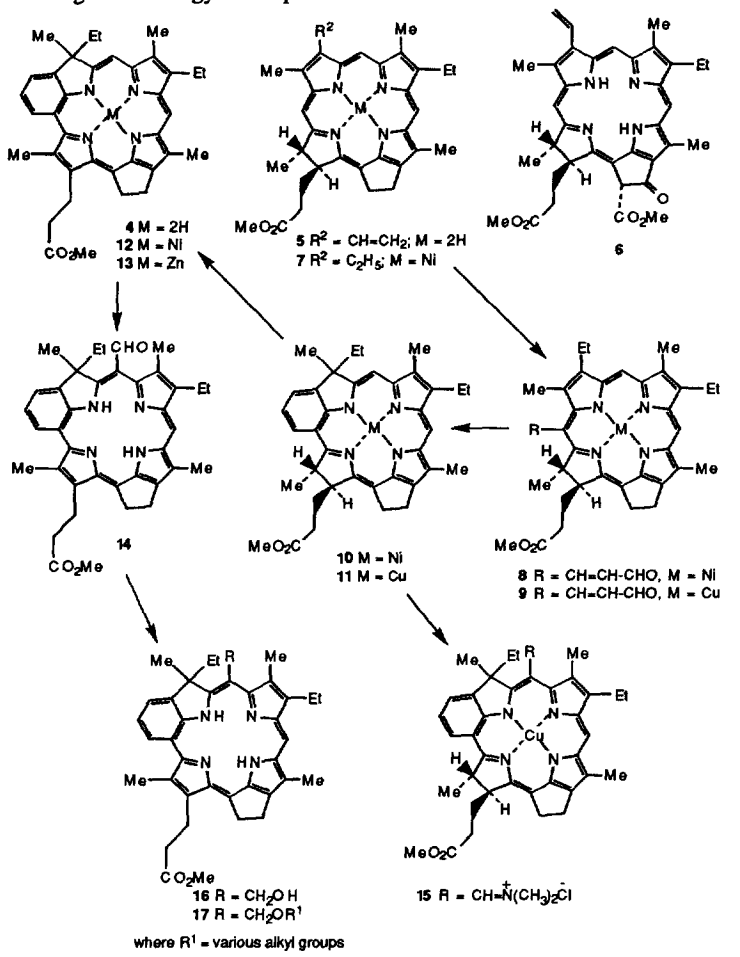
With few exceptions,[8] most of the benzochlorin derivatives prepared so far are based on symmetrical porphyrins, usually related to OEP. Besides their insoluble nature in most useful injectable solvents, it is also difficult to chemically modify these OEP-derived molecules. If unsymmetrical porphyrins are used as substrates and subjected to the reaction conditions discussed above, a mixture of isomers invariably results. In order to avoid this problem, we report here an efficient regioselective synthesis of benzochlorin analogues, e.g. **4**,[9] from methyl 9-deoxypyropheophorbide-a **5**, which

in turn was obtained in excellent yield from methyl pheophorbide-a **6**.[10] Methyl pheophorbide-a **6** was isolated from *Spirulina pacifica* by following methodology developed earlier in our laboratories.[11]



For the preparation of benzochlorin **4**, the nickel(II) complex **7** of methyl 9-deoxymesopyropheophorbide-a was reacted with $POCl_3$ and 3-N,N-dimethylaminoacrolein;[3] the 2-formylvinyl analogue **8** thus obtained was cyclized with TFA or H_2SO_4 and the benzoisobacteriochlorin **10** was produced in 40% yield. Attempts were made to convert isobacteriochlorin **10** into benzochlorin **12** by reacting with DDQ or chloranil, but failed. Removal of the metal and then treatment with DDQ gave the desired benzochlorin **4** in 34% yield. As shown in Figure 1, sensitizer **4** features a long wavelength absorption at 708 nm. To our surprise, when **4** was converted into its zinc(II) complex **13**[12] it showed a strong red-shifted absorption at 753 nm (ε 32,000). The nickel(II) complex **12** showed a red shift in its absorption spectrum, and displayed a strong absorption at 738 nm, and the long wavelength absorption of benzoisobacteriochlorin **10** was observed at 656 nm. Osuka et al.[13] have recently reported monomeric and dimeric benzochlorins with long-wavelength absorptions at about 672 nm.

Further spectroscopic studies and other physiochemical properties (e.g. singlet oxygen production with **13** and other metal complexes) are in progress.

In our efforts to understand the structure-activity relationships among various sensitizers, we have previously

prepared a series of alkyl ether analogues of pyropheophorbide-a[14] and so-called "benzoporphyrin derivatives".[15] Among such derivatives, in both series, the n-hexyl and n-heptyl ether derivatives were found to be extremely effective for *in vivo* photosensitizing activity compared to their parent analogues or other ether derivatives. To understand the generic requirement(s) among various photosensitizers, we intended to prepare a series of alkyl ether analogues of benzochlorin **4**. For the synthesis of these compounds, the nickel(II) complex **12** was reacted with the Vilsmeier reagent prepared from $POCl_3$/DMF. The resulting formyl derivative was demetalated upon treatment with H_2SO_4 and after oxidation the meso-formylbenzochlorin **14**[16] was isolated in 76% yield; it showed a pronounced long wavelength absorption at 735 nm (Figure 1). The zinc(II) complex of meso-formylbacteriochlorin **14** showed a strong absorption at 759 nm. By following a similar approach, the isobacteriochlorin copper(II) complex **11** was converted into the iminium salt **15**, which featured a strong absorption at 723 nm. Thus, compared with sensitizer **11**, a red shift of 63 nm was observed. Reaction of the formylbenzochlorin **14** with sodium borohydride gave the hydroxymethyl analogue **16** in excellent yield; upon reaction with HBr/AcOH and then with a series of alcohols, the corresponding alkyl ether derivatives **17** were obtained in 70-75% yields.

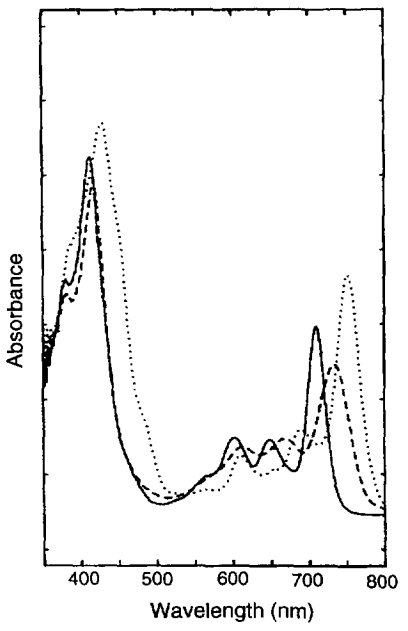


Figure 1: Optical spectra, in dichloromethane, of: (——) benzochlorin **4**; (·····) zinc(II) benzochlorin **13**; and (- - - -) formylbenzochlorin **14**.

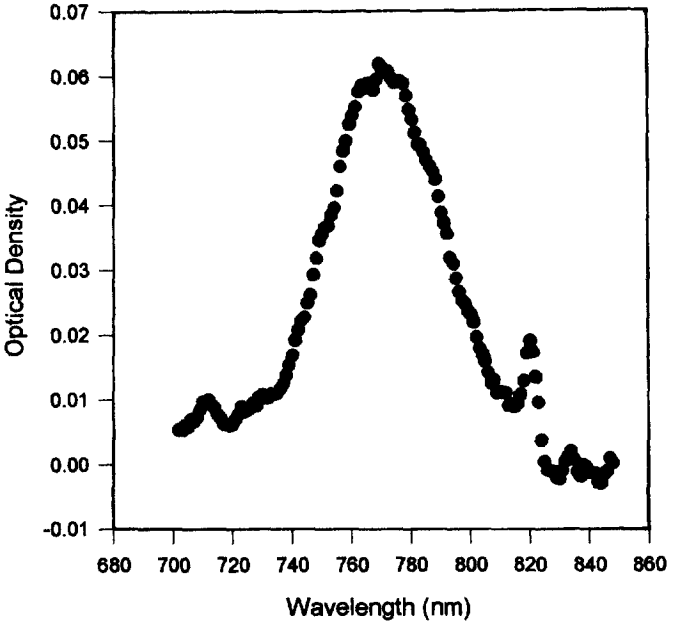


Figure 2: In vivo absorption spectrum of **13** in the colon 26 tumor in a Balb-C mouse. The spectrum was taken 23 h post-injection of **13**, i.v. at 5.9 μmoles/Kg mouse. The vertical axis is the absorbance in conventional log units. The peak at 820 nm is an artifact caused by the Xenon arc lamp of the spectrometer.

In Vivo Reflection Spectroscopy: The absorption spectra of a compound in living tissues can be recorded using an instrument and technique which we have developed.[17] In brief, the experiment measures the light scattered through the tissue. The non-invasive character of this measurement makes data collection possible at a number of time points after the i.v. injection of a potential photosensitizer.

For a sensitizer to be useful in PDT, it has to be retained in tumors for a longer time than in normal cells. *In vivo* drug absorption spectroscopy enables us to investigate: (i) the shift in the absorption spectrum of sensitizers (*in vitro* vs.

in vivo), and (ii) the uptake and clearance characteristics of a photosensitizer. The *in vivo* spectrum of zinc(II) benzochlorin **13** is shown in Figure 2; here it can be seen that the sensitizer **13** remains in the tumor for at least 23 h and shows a red shift of 14 nm in tissue (to 770 nm), compared with its *in vitro* absorption (753 nm/CH_2Cl_2). These results are of great value for evaluating and understanding the efficacy of a new sensitizer. From Figure 2 it can be determined that: (a) 23 h post-injection of the drug the photosensitizer is retained in the tumor, and thus these tumors can be treated even at 24 h post injection of the drug; (during this time period, it is likely that most sensitizers will clear from the surrounding tissues), and (b) the tumor should be treated with light at 770 nm, i.e. at the sensitizer's *in vivo* absorption maximum, instead of 756 nm (its *in vitro* absorption maximum). The synthesis, uptake, clearance, and *in vivo* biological character-istics of other sensitizers related to benzochlorins **4, 13, 14, 15, 17** and various metal complexes are currently in progress, and will be reported in our full paper.

The structures of new compounds were confirmed by NMR and mass spectroscopy.

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8. For benzochlorins from deuteroporphyrin-IX dimethyl ester, see compounds **12** and **13** in reference 3.
9. Note that "benzochlorin" analogues prepared *from* chlorins should correctly be termed "benzoisobacteriochlorins" (interrupted conjugation in adjacent rings) or "benzobacteriochlorins" (interrupted conjugation in opposite rings). Selective dehydrogenation of the "chlorin" subunit in either case will then afford "benzochlorins".
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12. ^{1}H NMR spectrum of **13**: (δ, ppm) 9.63 (m, 1H, benzo-H); 8.60, 8.24 (each s, 1H, meso-H); 8.12 (m, 2H, benzo-H); 4.12 (m, 4H, $C\underline{H}_2CH_2CO_2Me$ and $C\underline{H}_2CH_3$ ring B); 3.75, 3.46, 3.25, 3.12 (each s, 3H, Me and OMe rings B, C, and D); 3.7-3.2 (m. 8H, $CH_2CH_2CO_2Me$, $C\underline{H}_2CH_3$ ring B and CH_2CH_2 ring E); 2.30 (q, 2H, $C\underline{H}_2CH_3$ ring A); 2.00 (s, 3H, Me ring A); 1.60 (t, 3H, $CH_2C\underline{H}_3$ ring B); 0.12 (t, 3H, $CH_2C\underline{H}_3$ ring A).
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16. ^{1}H NMR spectrum of **14**: (δ, ppm) 11.10 (s, 1H, CHO); 8.88 (m, 1H, benzo-H); 8.25 (s, 1H, meso-H); 7.80 (m, 2H, benzo-H); 4.10 (m, 4H, $C\underline{H}_2CH_2CO_2Me$ and $C\underline{H}_2CH_3$ ring B); 3.75, 3.12, 2.80, 2.60 (each s, 3H, Me and OMe rings B, C, and D); 1.98 (s, 3H, Me ring A); 1.50 (t, 3H, $CH_2C\underline{H}_3$ ring B); 0.10 (t, 3H, $CH_2C\underline{H}_3$ ring A).
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