Synthesis and Spectroscopic Properties of Novel Benzochlorins Derived from Chlorophyll a

Sashikumar Mettath,[†] Masayuki Shibata,[‡] James L. Alderfer,[‡] Mathias O. Senge,[§] Kevin M. Smith,[∥] Robert Rein,[‡] Thomas J. Dougherty,[†] and Ravindra K. Pandey*,[†]

Chemistry Division, Department of Radiation Biology, Division of Radiation Medicine, and Department of Biophysics, Roswell Park Cancer Institute, Buffalo, New York 14263, Institute für Organische Chemie, Frie Universitãt, Berlin, Germany, and Department of Chemistry, University of California, Davis, California 95616

Received October 27, 1997

Methylpheophorbide-*a* (a chlorophyll *a* analog) was converted into methyl 13^1-deoxypyropheophorbide-*a* **2** in excellent yield. The Ni(II) complex of **2** on hydrogenation and subsequent Vilsmeier reaction with phosphorus oxychloride and 3-(*N*,*N*-dimethylamino)acrolein produced Ni(II) methyl 20-(2-formylvinyl)-13^1-deoxypyropheophorbide-*a* **4**. In contrast to other synthetic and naturally occurring isobacteriochlorins, the isobacteriochlorin obtained by acid-catalyzed cyclization of **4** was found to be unstable and oxidized to the corresponding benzochlorin. The free base benzochlorin **7**, obtained by demetalation, up on DDQ/methanol treatment produced a mixture of 13^2-oxo- and 13^1-methoxy-13^2-oxobenzochlorins **8**, **9**. Surprisingly, under similar reaction conditions, an ethanolic solution of DDQ afforded 13^1,13^2-dioxobenzochlorin **13** as a major product. Vilsmeier reaction ($POCl_3$/DMF) of Ni(II) benzochlorin **6** gave unexpected 5-formyl-13^2-oxobenzochlorin **15**. Zn(II) complexes of the newly synthesized benzochlorins showed long wavelength absorptions in the range of 750−753 nm. Thus, compared to the respective free base analogues bathochromic shifts of 40−42 nm were observed. Despite extensive studies in benzochlorins, these are the first examples which exhibit such remarkable long wavelength absorptions in their electronic absorption spectra. The structures of novel benzochlorins were confirmed by extensive NMR [2D NMR (ROESY), ^{13}C NMR] and X-ray crystallographic studies. On the basis of the crystal structure of oxobenzochlorin **8**, the chemical reactivity of other benzochlorin analogues were examined by semiempirical molecular orbital theory. Our results are in agreement with the previous qualitative electron sextet hypothesis proposed for chlorin systems by Woodward. The fluorescence quantum yields and the singlet oxygen yields of the free base and Zn(II) benzochlorins were measured relative to tetraphenylporphyrin (TPP) in benzene.

Introduction

One of the most important uses of porphyrins is their application in photodynamic therapy (PDT) of various cancers.[1] An important characteristic of certain porphyrins is their ability to localize selectively in certain types of tumors which allow their selective destruction upon irradiation with light.[2] Currently, Photofrin, a mixture of porphyrin oligomers, is the only drug which has been approved worldwide for the treatment of various types of cancers. Despite the utility of Photofrin, it has several disadvantages. It is a complex mixture of oligomers linked with ether and ester linkages,[3] which makes it difficult to examine the molecular basis of its PDT activity. Also, it remains in the skin and other normal tissues for a long time and induces cutaneous phototoxicity. Finally due to its absorbance at 630 nm, where tissue penetration is not optimum, the size of tumors that can be treated is limited.[4] It is desirable therefore, to develop photosensitizers with strong absorption at wavelengths near or above 700 nm to take full advantage of greater tissue penetration.

In recent years a number of so-called second generation photosensitizers related to chlorins, bacteriochlorins, azaporphyrins, porphycenes, and expanded porphyrins have been reported in the literature.[5] Among these novel compounds, benzochlorins have been studied in depth by various investigators due to their ability to localize in

* Corresponding author: Phone: (716) 845-3203. Fax: (716) 845-8920. e.mail: PDT CTR@SC3101.MED.BUFFALO.EDU.
[†] Chemistry Division, Roswell Park Cancer Institute.
[‡] Department of Biophysics, Roswell Park Cancer Institute.
[§] Frie Universität.
[∥] University of California.

(1) (a) Dougherty, T. J.; Marcus, S. C. *Eur. J. Cancer* **1992**, *28A (10)*, 1734. (b) Gomer, C. J.; Rucker, N.; Ferrario, A.; Wang, S. *Radiat. Res.* **1989**, *120*, 1.

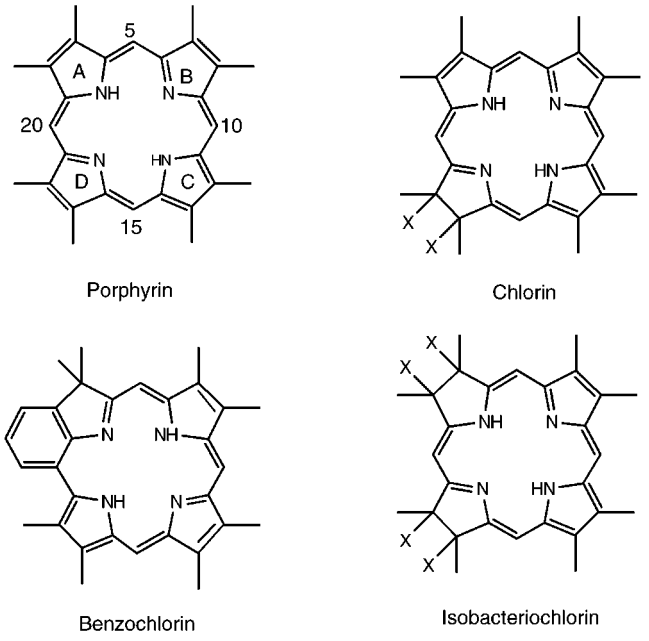
(2) Weishaupt, K. R.; Gomer, C. J.; Dougherty, T. J. *Cancer Res.* **1976**, *1*, 1.

(3) (a) Pandey, R. K.; Dougherty, T. J. and Smith, K. M. *Tetrahedron Lett.* **1988,** *29*, 4657. (b) Kessel, D.; Thompson, P.; Musselman, B. and Chang, C. K. *Photochem. Photobiol.* **1987**, *29*, 4657. (c) Morris, I. K.; Ward, A. D.; *Tetrahedron Lett.* **1988**, *29*, 2501. (d) Scourides, P. A.; Bohmer, R. M.; Kaye, A. H.; Morstyn, G. *Cancer Res.* **1987**, 47, 3439. (e) Pandey, R. K.; Shiau, F. Y.; Medforth, C. L.; Dougherty, T. J.; Smith, K. M. *Tetrahedron Lett.* **1990**, *5*, 1990. (f) Berenbaum, M. C.; Bonnett, R.; Scourides, P. A. *Br. J. Cancer* **1982**, *45*, 571. (g) Byrne, C. J.; Marshallasy, L. V.; Ward, A. D. *J. Photochem. Photobiol., B* **1990**, *6*, 13. (h) Byrne, C. J.; Morris, I. K.; Ward, A. D. *Aust. J. Chem.* **1990**, *43*, 1889. (i) Pandey, R. K.; Shiau, F. Y.; Medforth, C. J.; Dougherty, T. J.; Smith, K. M. *Tetrahedron Lett.* **1990**, *31*, 789. (j) Shiau, F.-Y.; Pandey, R. K.; Ramaprasad S.; Dougherty, T. J.; Smith, K. M. *J. Org. Chem.* **1990**, *55*, 2190. (k) Pandey, R. K.; Smith, K. M.; Dougherty, T. J. *J. Med. Chem.* **1990**, *33*, 2032. (l) Pandey, R. K.; Shiau, F.-Y.; Dougherty, T. J.; Smith, K. M. *Tetrahedron* **1991**, *47*, 9571. (m) Kessel, D.; Thomson, P.; Musselman, B.; Chang, C. K. *Cancer Res.* **1987**, *47*, 4642. (n) Pandey, R. K.; Dougherty, T. J. *Cancer Res.* **1989**, *49*, 2042. (o) Pandey, R. K.; Siegel, M. M.; Tsao, R.; McReynolds, R.; Dougherty, T. J. *Biomed. Environ. Mass Spectrom.* **1990**, *19*, 405.

(4) Bonnett, R.; Berenbaum, M. Porphyrins as photosensitizers. *Ciba Found. Symp. 146*, **1989**, 40 and references therein.

S0022-3263(97)01968-3 CCC: $15.00
Published on Web 02/18/1998

Scheme 1. Basic structure of porphyrin and reduced porphyrins



tumors and cause tumor necrosis after treating with laser light at their long wavelength absorptions. Basic structures of porphyrins and reduced porphyrins are shown in Scheme 1.

Starting from octaethylporphyrin (OEP), the preparation of benzochlorin was first reported by Johnson's group in 1978.[6] The utility of this class of compounds as photosensitizers for PDT was later shown by Morgan et al.[7] in mice tumor models. With few exceptions, most of the benzochlorin derivatives prepared by Morgan and coworkers are based on symmetrical porphyrins, usually related to OEP. These compounds are difficult to evaluate for their in vivo efficacy due to their insoluble nature in various injectable vehicles.

One of the major problems associated with benzochlorin preparation is the difficulty in demetalation at the final step of the synthesis. A few years ago, Gunter et al.[9a] converted a series of Ni(II) 5,15-diarylporphyrins to the corresponding benzochlorins, which on acid treatment produced the corresponding free base analogues in excellent yield. The X-ray analyses of Ni(II) 5,15-diarylbenzochlorins showed a remarkable distortion in the ring, which possibly helped in the removal of the central metal ion. This methodology was later extended by Osuka et al.[9b] for preparing related *meso*-substituted analogues.

Vicente and Smith[10] were the first to show the utility of 3-(*N*,*N*-dimethylamino)acrolein for introducing (2-formylvinyl) substituents directly at the *meso*-positions of the metalated porphyrin and chlorin systems. Under strong acidic conditions, nickel(II) complexes were then converted into the corresponding benzochlorin, isobacteriochlorn, and bacteriochlorin analogues. Unfortunately, the conversion of the metalated analogues into the corresponding free bases at the last step of the synthesis was unsuccessful.

To explore the utility of this class of compounds as photosensitizers, we were interested in developing an efficient route for the preparation of benzochlorin analogues from the readily available starting material. In one approach, methyl 13[1]-deoxypyropheophorbide-*a* chlorophyll *a* based chlorin was used as a substrate for the following reasons: (i) The 2-formylvinyl substituent, a key functional group required for benzochlorin preparation, can be regioselectively introduced at the δ *meso*-position of the macrocycle. In fact, this strategy was based on the previously demonstrated report that aromatic electrophilic substitutions occur adjacent to the reduced ring in chlorin systems.[11] (ii) From the X-ray crystal structure data, it has also been shown that the presence of a five-membered fused ring in Ni(II) complexes of pheophorbide analogues show significant distortion[12] in the molecules, which we thought would be enhanced by the presence of another fused six-membered ring and might help in demetalation at the final step. In this paper, we describe our attempts to achieve some novel free base benzochlorins, which were otherwise difficult to obtain by following the conventional methods. Furthermore, modification of these benzochlorins would generate a series of related analogues as effective photosensitizers for photodynamic therapy.

Results and Discussion

Chlorophyll *a* was extracted from *Spirulina pacifica* and converted into methyl pheophorbide-*a* by following the improved method developed in our laboratories.[13] Reaction of methyl pheophorbide-*a* with 2,4,6-collidine at refluxing temperature produced methyl pyropheophorbide-*a* **1** in >85% yield.[14] In tetrapyrrole chemistry it has been shown by us and others that the Vilsmeier reagents react with enolizable ketones to give α,β-unsaturated chloroaldehydes and other byproducts.[15] Thus, to avoid these side reactions, the keto-group was reduced by reacting with $NaBH_4$/TFA[16] and the corresponding 13[1]-deoxypyropheophorbide-*a* **2** was isolated in 75% yield (Scheme 2). Chlorin **2** was then converted into the corresponding Ni(II) derivative on refluxing with Ni(acac)$_2$/*o*-xylene solution under nitrogen atmosphere. To eliminate the formation of undesirable byproducts under Vilsmeier reaction conditions,[17] the vinyl group present on ring A of chlorin **2** was replaced with an ethyl

(5) For reviews see: (a) Bonnett, R. *Chem. Rev.* **1995**, *24*, 19. (b) Sessler, J. L.; Hemmi, G.; Mody, T. K.; Murai, T.; Burrell, A.; Young, S. W. *Acc. Chem Res.* **1994**, *27*, 43. (c) Pandey, R. K.; Majchrzycki, D. F.; Smith, K. M.; Dougherty, T. J. *Proc. SPIE−Int. Soc. Opt. Eng.* **1989**, *1065*, 164.

(6) Arnold, D. P.; Holmes, R. G.; Johnson, A. W.; Smith, A. R. P.; Williams, G. A., *J. Chem. Soc., Perkin Trans. 1* **1978**, 1660.

(7) Morgan, A. R.; Noris, S.; Rampersaud, A. *Proc. SPIE−Int. Soc. Opt. Eng. 847*, 166.

(8) Morgan, A. R.; Skalkos, D.; Maguire G.; Rampersaud, A.; Garbo, G.; Keck, R.; Selman, S. H. *Photochem. Photobiol.* **1992**, *55*, 133.

(9) (a) Gunter, M. J.; Robinson, B. C.; Gulbis, J. M.; Tiekink, R. T. *Tetrahedron* **1991**, *47*, 7853. (b) Osuka, A.; Yoshiya, I.; Maruyama, K. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 3322.

(10) Vicente, M. G. H.; Smith, K. M. *J. Org. Chem.* **1991**, *56*, 4407.

(11) Woodward, R. B.; Skaric, *J. Am. Chem. Soc.* **1961**, *83*, 4676.

(12) (a) Senge, M. O.; Smith, K. M. *Acta Crystallogr.* **1997**, *53*, 1314. (b) Senge, M. O.; Smith, K. M. *Photochem. Photobiol.* **1991**, *54*, 841 and *Photochem Photobiol.* **1994**, *60*, 139.

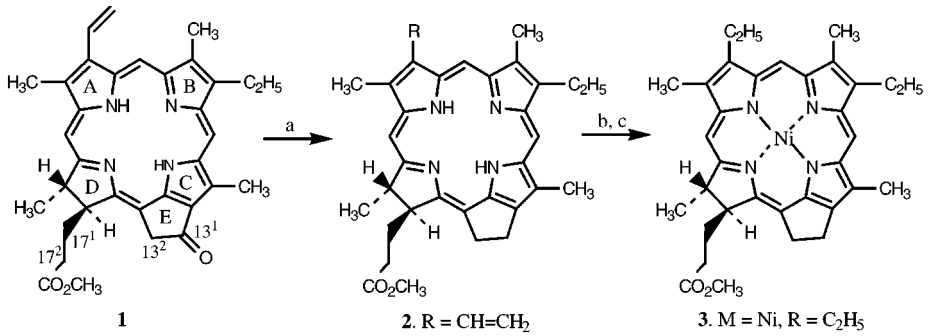
(13) (a) Smith, K. M.; Goff, D. A.; Simpson, D. J. *J. Am. Chem. Soc.* **1985**, *107*, 4946. (b) Pandey, R. K.; Bellnier, D. A.; Smith, K. M.; Dougherty, T. J. *Photochem. Photobiol.* **1991**, *53*, 65.

(14) Smith, K. M. In *Porphyrins and Metalloporphyrins*; Smith, K. M., Ed.; Elsvier: Amsterdam, 1975.

(15) (a) Pandey, R. K.; Constantine, S.; Goff, D. A., Kozyrev, A. N., Dougherty, T. J.; Smith, K. M. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 105. (b) Wray, V.; Jürgens, U. Brockmann, H., Jr. *Tetrahedron* **1979**, *35*, 2275.

(16) (a) Gribble, G. W.; Leese, R. M.; Evans, B. E., *Synthesis* **1978**, 763. (b) Smith, N. W.; Smith, K. M. *Energy and Fuels* **1990**, *4*, 675. (b) Jeandon, C.; Ocampo, R.; Callot, H. J. *Tetrahedron Lett.* **1993**, *34*, 1791.

Scheme 2.[a] **Preparation of methyl 2-devinyl-2-ethyl-13[1]-deoxypyropheophorbide-*a***



[a] Reagents: (a) $NaBH_4$/TFA; (b) $Ni(acac)_2$ (c) H_2/10% Pd−C.

functionality to yield **3** by hydrogenation using 10% Pd/C as a catalyst. Subsequent reaction with 3-(*N,N*-dimethylamino)acrolein regioselectively introduced the 2-formylvinyl substituent at the δ-meso-position, and the corresponding methyl 13[1]-deoxymesopyropheophorbide-*a* **4** was obtained in 75% yield. Our synthetic strategy for the cyclization of formyl derivative **4** to the respective benzochlorin was based on the approach of Arnold and co-workers.[6] Thus, the 2-formylvinyl chlorin **4** was reacted under various acidic reaction conditions (such as PTS, TFA, BF_3).[18] It was expected that the reaction of **4** will produce isobacteriochlorin **5**, which on demetalation and subsequent DDQ treatment would generate free base benzochlorin **7**. In our initial approach, the intermediate product obtained after cyclization of formylvinyl chlorin **4** was not isolated but was reacted with sulfuric acid and then with methanolic DDQ. After the standard workup, the reaction mixture was purified by column chromatography, and the two main products were isolated, having long wavelength absorptions at λ_{max} 711 and 708 nm, respectively. The mass spectrum of the faster moving band with a long wavelength of absorption at 711 nm showed a molecular ion peak at *m/z* 586, which was 14 D higher than the expected benzochlorin **7**. The ^{1}H NMR spectrum showed the resonance of a total of 38 protons, 2 protons less than required for benzochlorin **7**. The presence of two singlets and two multiplets pertaining to five protons (two *meso* and three benzyl ring protons), indicated the presence of a fused six-membered ring. The ^{1}H NMR also showed a singlet for two protons at 3.90 ppm instead of the expected doublet pattern for 13[1] and 13[2] methylene protons. The mass spectrum and the ^{1}H NMR spectrum thus indicated the presence of a keto-group either at the 13[1] or 13[2] position. The formation of such oxo-analogues has also been reported by Smith et al.[19] in their attempts to convert methyl pyropheophorbide-*a* into phytoporphyrin methyl ester on reacting with various oxidizing agents. Thus, there was a possibility that the intermediate product **7** could generate a mixture of dioxo-chlorin and its possible mono oxo-derivatives in which the keto-group is present either at the 13[1] or 13[2] position. However, due to the benzylic nature of position 13[2], it is more prone to oxididation. Thus, the structure for this compound was initially assigned as **8**. The second product from the mixture which was more polar (λ_{max} 708 nm) showed a molecular ion peak at *m/z* 616. Thus, compared to the expected benzochlorin **7** (*m/z* 572), an excess value of 44 Da were observed. The ^{1}H NMR spectrum (400 MHz) showed a complex pattern for certain resonances. There was a singlet (for one proton) at 5.97 ppm and six singlets for methyl protons (3.1−3.8 ppm), indicating an extra methyl resonance. The expected doublet pattern for 13[1] and 13[2] methylene protons was also missing. However, the ^{1}H spectrum of this compound at 600 MHz clarified the complex pattern, and a set of doublets was observed (for details see the Experimental Section). On the basis of these data, the structure of this product was assigned as **9**.

To define the structure of the compounds obtained at every step of the synthesis, we purified the reaction product obtained after acid cyclization of (formylvinyl-)chlorin **4**. The NMR spectrum of the isolated product showed two protons less than expected for the desired isobacteriochlorin **5**. For isobacteriochlorin **5**, the methyl group on ring D was expected to be a doublet (due to the presence of the hydrogen attached to the same carbon) but was observed as a singlet with a downfield shift, and the resonances for 17-H and 18-H protons were missing. These results indicated that ring D had been oxidized. This was also confirmed by mass spectrometry (molecular ion peak at 628 Da, i.e., 2 Da less than expected for isobacteriochlorin **5**). These data confirm that under the experimental conditions used, the intermediate isobacteriochlorin **5** was not stable and immediately oxidized to the corresponding benzochlorin **6**. These findings were in contrast to the previous report[10] in which certain chlorins such as octaethyl chlorin (OEC) and mesochlorin e_6 trimethyl ester were smoothly converted to their respective isobacteriochlorins. Ni(II) benzochlorin **6** on treatment with TFA at room temperature gave the corresponding free base analogue **7** in excellent yield. To determine the reactivity of **7** with oxidizing agents, it was treated with methanolic DDQ. As expected, the purification of the reaction mixture generated mainly two products, which were found to be identical to benzochlorin **8** and **9** by visible, ^{1}H NMR, and mass spectrometry analyses.

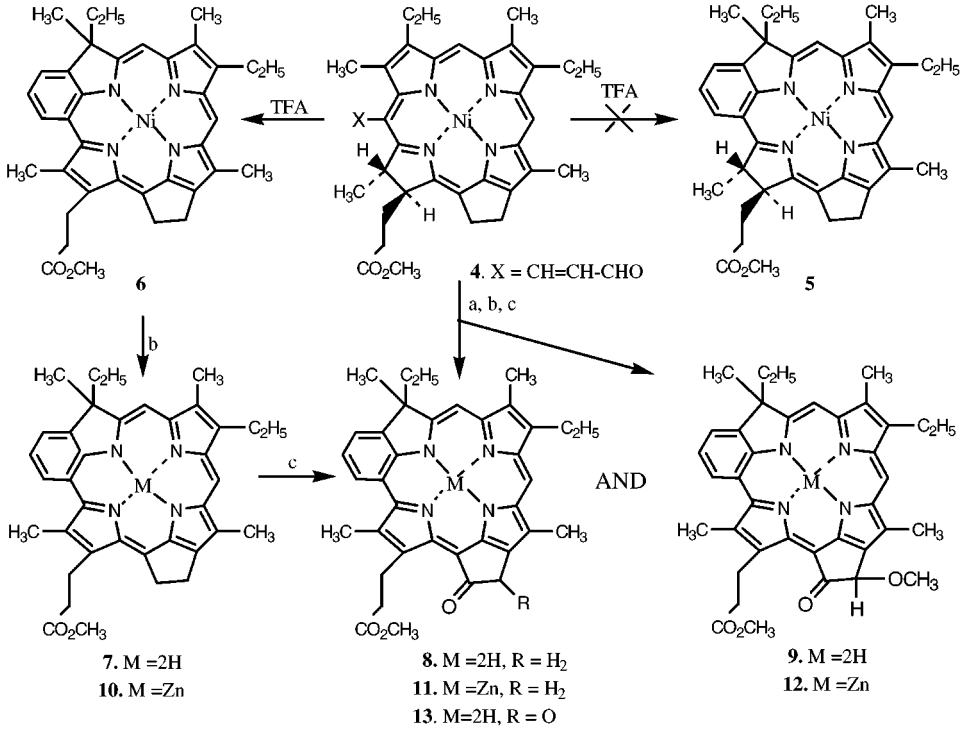
The combination of NMR and mass spectral data for compound **9** indicated the presence of an oxo- and a methoxy group individually substituted either at position 13[1] or 13[2]. Hence in order to ascertain the position of the oxygen, 2D NMR (ROESY) experiments were performed. The starting point of focus was the two ethyl groups on ring A and ring B. The CH_2 protons of the ethyl group on ring A are attached to a sp^3 carbon, but

(17) (a) Nichol, A. W. *J. Chem. Soc. C* **1970**, 903. (b) Smith, K. M.; Bisset, G. M. F.; Bushell, M. J. *J. Org. Chem.* **1980**, *45*, 2218.

(18) Tetrafluoroacetic acid (TFA) gave the cyclized product in a reasonably good yield.

(19) Kenner, G. W.; McCombie, S. W.; and Smith, K. M. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2517.

Scheme 3.[a] Preparation of free base and metallated benzochlorins and their oxo-analogs



[a] Reagents: (a) TFA; (b) H_2SO_4; (c) DDQ/MeOH.

the other CH_2 protons of the ring B ethyl substituent are attached to a sp^2 carbon atom. Therefore, the CH_2 protons on ring A are expected to be upfield with respect to the CH_2 protons of ethyl group attached on ring B. Thus, the resonances at 2.67 ppm, integrating for two protons, are assigned to the CH_2 protons of ring A, and resonances at 3.59 ppm to the CH_2 protons on ring B. All other assignments were made on the basis of these resonances. As shown in Figure 1, a strong interaction was observed between the methyl protons on ring C (s, 3.23 ppm) and the methyl protons at 3.71 ppm, and a singlet for one proton at 5.90 ppm. Since the methyl protons (3.23 ppm) are on ring C, the protons resonating at 3.71 and 5.90 ppm are assigned to the methoxy group and the CH protons at position 13^1 of the five-membered ring. There exists a strong interaction between the methyl group (3.36 ppm) on ring D and the methylene protons (4.28 ppm) of the propionic ester (pme) group. However, no interaction was observed between the methylene protons of the pme group (4.28 ppm) and the protons of the five-membered ring. The interactions of various protons observed for this compound were also compared to the ROESY assignments of porphyrin **19** (Scheme 4). On the basis of these results, the structure for the more polar product was assigned as 13^1-methoxy-13^2-oxobenzochlorin **9**.

The structure of benzochlorin **8** was also confirmed by 2D ROESY NMR studies and X-ray crystallography (Figure 2).

In our preliminary in vivo studies with free base and the related Zn(II) analogue of benzochlorins derived from OEP (**20** and **21**, Scheme 5), the metalated analogue showed enhanced tumor uptake with similar PDT efficacy.[20] Compound **21** was also evaluated for skin phototoxicity. Interestingly, compared to Photofrin, benzochlorin **21** showed reduced skin phototoxicity at the therapeutic dose. These results were encouraging since

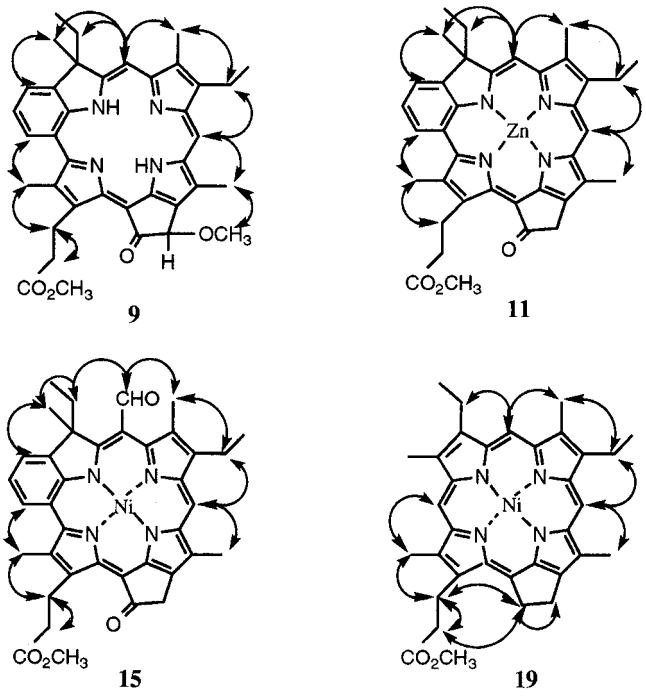
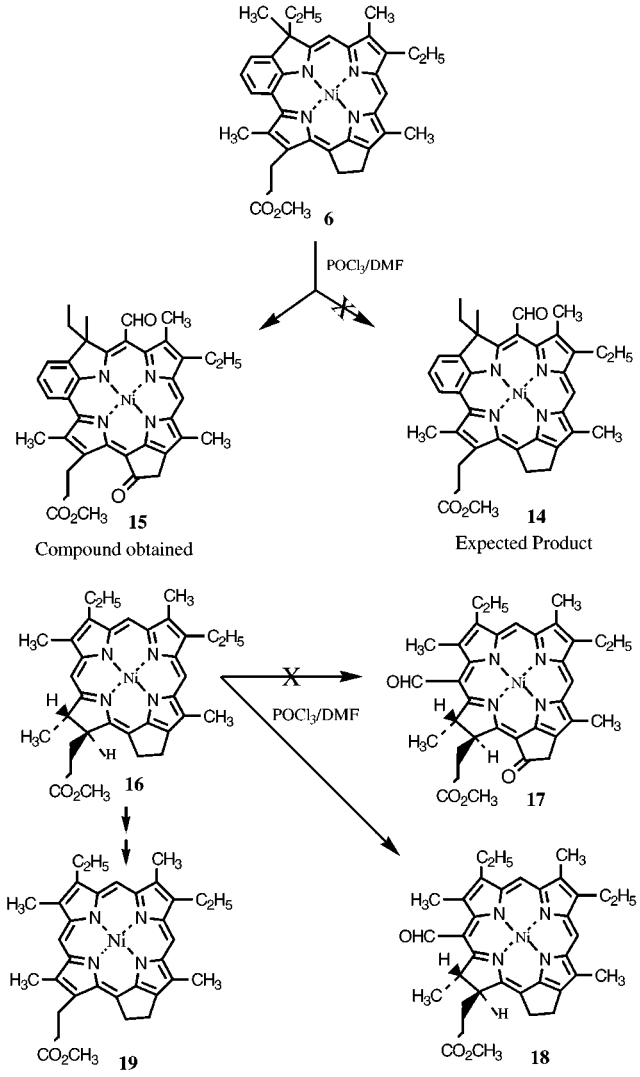


Figure 1. Interaction observed between various protons by 2D ROSEY NMR studies.

phototoxicity is one of the major drawbacks of Photofrin. To investigate the spectroscopic and therapeutic properties of metalated analogues of the newly synthesized chlorins, benzochlorins **7** and **8** were converted into the corresponding Zn(II) complexes in quantitative yields. These benzochlorins **11** and **12** exhibited long wavelength absorptions at λ_{max} 753 and 750 nm, respectively. Thus, compared to free base analogues **7** and **8**, a shift of 42 nm was observed. Despite extensive studies in benzochlorin chemistry these are the first examples in which

Scheme 4. Vilsmeier reaction products of Ni(II) complexes of benzochlorin 6 and methyl 2-devinyl-2-ethyl-13[1]-deoxypyropheophorbide-*a* 16



such a remarkable long wavelength absorption has been found. The structures of **11** and **12** were confirmed by NMR (^{13}C NMR, 2D NMR, NOE's) studies. Herein, we present the 2D ROESY studies of compound **11**. The assignments of various substituents was done on the basis of the resonances of the two ethyl groups attached on ring A and B. The CH_2 protons of ethyl group on ring A are attached to a sp^3 carbon, and the CH_2 protons of ethyl group on ring B are attached to a sp^2 carbon atom. The CH_2 protons on ring A are expected to be upfield of CH_2 resonances of the ethyl group present on ring B. Thus, the protons resonating at 2.67 ppm were assigned to the CH_2 protons of the ethyl group on ring A. These protons had strong interaction with the protons resonating at 2.14 ppm (s, three protons), 8.24 ppm (*meso*-proton), and a weak interaction with protons resonating at 0.13 ppm (three protons). On the basis of these data, these resonances were assigned to CH_3 group at position-3, *meso*-proton at position 5, and the CH_3 protons of the ethyl group present on ring A, respectively. The 5-*meso*-proton had strong interaction with protons at 3.13 ppm which were assigned to the methyl group at position-7,

(20) In preliminary studies, the Zn(II) benzochlorin **21** showed better tumor uptake than the corresponding free base analogue, without any significant difference in photosensitizing efficacy. At a dose of 5.0 mg/kg, mice (10 mice/group) transplanted with SMT-F tumors were treated with light (135 J/cm^2) at their long wavelength absorptions for 30 min after 24 h postinjection of the drug, and 80% tumor cure was observed (8 out of 10 mice were tumor free on day 30).

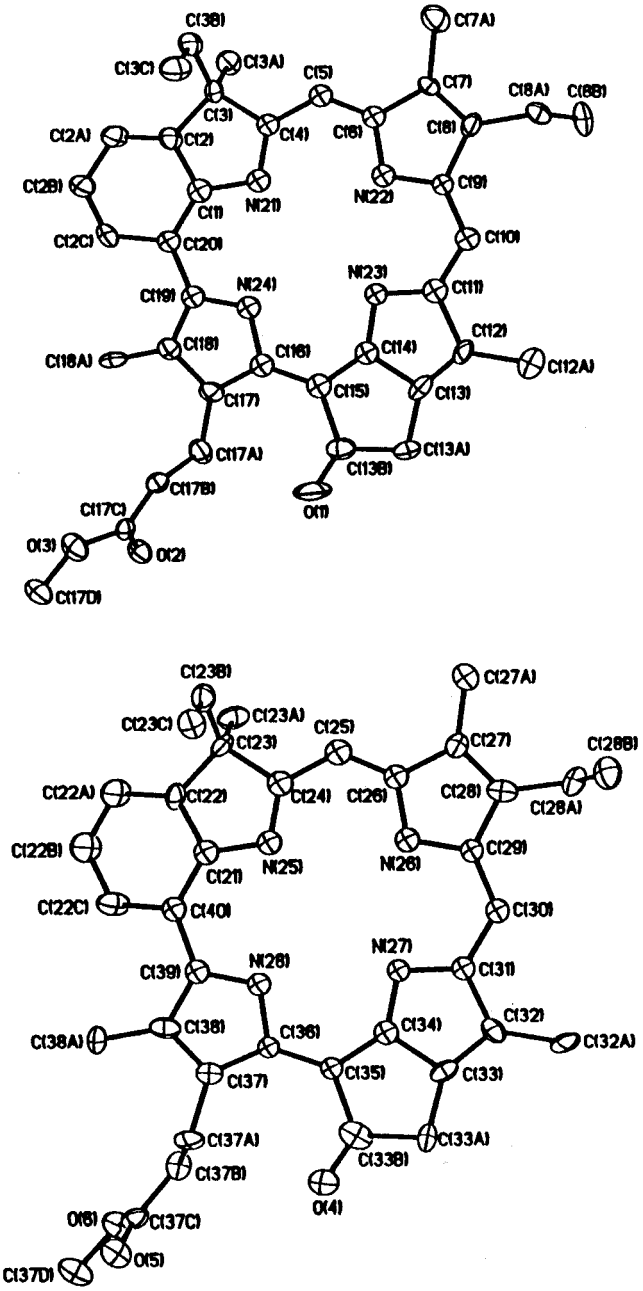
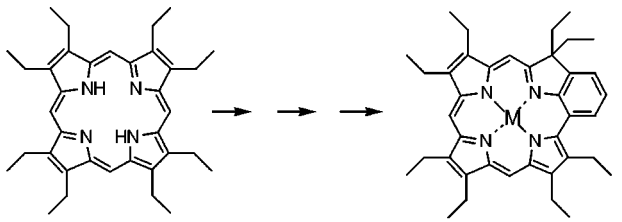


Figure 2. View of the two crystallographically independent molecules of benzochlorin **8** in the crystal. Hydrogen atoms have been omitted for clarity; ellipsoides show 50% occupancy.

Scheme 5. Structure of benzochlorins derived from OEP



which in turn had strong interaction with the CH_2 protons at 3.53 ppm, and CH_3 protons at 1.62 ppm. The resonances at 3.53 and 1.62 ppm were therefore assigned to CH_2 and CH_3 protons of the ethyl group present at position-8. The CH_2 protons at 3.53 ppm had strong interaction with the *meso*-protons at 8.44 ppm, which in turn had strong interaction with the methyl resonances observed at 2.80 ppm. The resonances that appeared at 2.80 ppm were therefore assigned to the methyl group present at position-12. No interaction was observed between the methyl protons at position-12 and the 13^1-CH_2 protons (ring E). The interactions observed for compound **11** were compared to the ROESY assignments obtained for porphyrin **19**, in which a weak interaction was observed between the methyl group at position 12 and the bridge protons at position 13^1. Further, a strong interaction was noticed between the protons at 13^1 and 13^2, as well as between 13^2-protons and protons present at positions 17^1 and 17^2. In compound **11** no such interaction was observed between the methylene protons (17^1 and 17^2) of ($CH_2CH_2CO_2CH_3$) and the protons of the five-membered ring. Had the protons of the ring been at position 13^2, a strong interaction should have been observed between these protons and the CH_2CH_2 protons of the 17-(2-(methoxycarbonyl)ethyl) group. On the basis of these results the resonances for two protons observed at 3.91 ppm as a singlet were assigned to the CH_2 protons present at position 13^1. One of the major products obtained by reacting **7** with methanolic DDQ was the methoxy derivative **9**, which we assumed was derived from the methanol used as a solvent. To our surprise, replacing methanol with ethanol as a solvent did not produce the expected ethoxy derivative, instead, an unexpected diketobenzochlorin **13** was isolated in 27% yield (Scheme 3).

A continuing objective of research in our laboratory has been to understand the structure−activity relationships among series of photosensitizers. To explore the utility of benzochlorins as useful photosensitizers in PDT, we were interested in preparing a series of analogues and evaluating them for their in vivo photosensitizing efficacy. Previously, studies with certain other types of photosensitizers have shown that lipophilicity of a molecule can play an important role in how they localize in tumor cells.[21−24] This was found to be true among various alkyl ether derivatives of pyropheophorbide-*a*. In these compounds the photosensitizing ability increased with the length of the carbon chain, being maximum in respective *n*-hexyl and *n*-heptyl analogues.[25] In our attempts to establish generic requirements for effective photosensitizers, we thought it worthwhile to prepare and evaluate a series of alkyl ether derivatives of the newly synthesized benzochlorin system. To achieve our goal, the Ni(II) benzochlorin **6** was reacted with $POCl_3$/DMF, and the formyl group was regioselectively introduced at the *meso*-position adjacent to the reduced ring. The formyl analogue with long wavelength absorption at λ_{max} 768 nm was purified by column chromatography and isolated in 35% yield. Mass spectrometry analysis gave a molecular ion peak at *m*/*z* at 614.28 Da, 14 Da more than the expected value for benzochlorin **14**. ^{1}H NMR data confirmed the presence of a formyl group at 11.05 ppm. However, the total number of protons were two proton less than expected, and the resonances for methylene protons generally observed as multiplets associated with positions 13^1 and 13^2 were missing. The ^{1}H NMR and the mass spectrum indicated the possibility of a keto group either at the 13^1 or 13^2 position. The structure of this compound was confirmed by 2D ROESY studies. The starting point of focus was the formyl group observed as a singlet at 11.05 ppm. All the other interactions that were observed are shown in Figure 1. The CH_2 protons of the ethylene group on ring B show interaction with the *meso*-proton (8.29 ppm), which in turn shows interaction with the methyl group on ring C. No interaction was observed between the methyl group on ring C and the methylene group of the five-membered ring (ring E). The protons on the phenyl ring (8.62 ppm) show stong interaction with the methyl group at 2.97 ppm. These protons were therefore assigned to the 18-methyl group, which in turn interacts with the methylene protons (3.96 ppm) of the 17-(2-(methoxycarbonyl)ethyl) group (17^1) attached to ring D. A strong interaction was also observed between the methylene protons (17^1 and 17^2 positions) of the propionic ester group. However, no interaction was observed between the CH_2 protons of pme and the methylene protons (4.04 ppm) associated with five-membered ring. The interactions that were observed for this compound were compared to the ROESY assignments of porphyrin **19** (Figure 1). A strong interaction was noted in **19** between the protons at position 13^2 and protons at 17^1 and 17^2 (CH_2 protons of pme). Compared to porphyrin **19**, in this product no interaction was observed between the ring E methylene protons (4.09 ppm) and the methylene protons of the pme group. Had this proton been at position 13^2, a strong interaction should have been observed. The absence of this interaction indicates that the resonances at 4.04 ppm are due to the CH_2 protons at position 13^1, and the keto group is regioselectively introduced at position 13^2. The proton and the 2D NMR spectrum of benzochlorin **15** are shown in Figure 3. These results were surprising, because under similar reaction conditions Ni(II) methyl 13^1-deoxypyropheophorbide-*a* **16** (lacking the fused phenyl ring) did not produce keto chlorin **17**. In contrast, the δ *meso*-formyl derivative **18** was isolated as a sole product.[26]

Our current efforts are concentrated in preparing the formylbenzochlorin **15** in larger quantity, which will then be converted into a series of alkyl ether analogues by following standard methodology.[25] The synthesis and biological data obtained from these compounds, other free

(21) Pandey, R. K.; Shiau, F. Y.; Sumlin, A. B.; Dougherty, T. J.; Smith, K. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1263.

(22) (a) Meunier, I.; Pandey, R. K.; Walker, M. M. Senge, M. O.; Dougherty, T. J.; Smith, K. M. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 1575. (b) Meunier, I.; Pandey, R. K.; Senge, M. O.; Dougherty, T. J.; Smith, K. M. *J. Chem. Soc., Perkin Trans. 1* **1994**, 961. (c) Pandey, R. K.; Potter, W. R.; Meunier, I.; Sumlin, A. B.; Smith, K. M. *Photochem. Photobiol.* **1995**, *62*, 764.

(23) Pandey, R. K.; Shiau, F.-Y.; Sumlin, A. B.; Dougherty, T. J.; Smith, K. M. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 491.

(24) (a) Pandey, R. K.; Jagerovic, N.; Ryan, J. M.; Dougherty, T. J.; Smith, K. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2615. (b) Pandey, R. K.; Jagerovic, N.; Ryan, J. M.; Dougherty, T. J.; Smith, K. M. *Tetrahedron* **1996**, *52*, 5349.

(25) (a) Pandey, R. K.; Sumlin, A. B.; Constantine, S.; Aoudia, M.; Potter, W. R.; Bellnier, D. A.; Henderson, B. W.; Rodgers, W. R.; Smith, K. M.; Dougherty, T. J. *Photochem. Photobiol.* **1996**, *64*, 194. (b) Henderson, B. W.; Bellnier, D. A.; Greco, W. R.; Sharma, A.; Pandey, R. K.; Vaughan, L. A.; Weishaupt, K. R.; Dougherty, T. J. *Cancer Res.* **1997**, *57*, 4000.

(26) Pandey, R. K.; Constantine, S.; Tsuchida, T.; Zheng, G.; Medforth, C. J.; Aoudia, A.; Kozyrev, A.; Rodgers, M. A. J.; Kato, H.; Smith, K. M.; Dougherty, T. J. *J. Med. Chem.* **1997**, *40*, 2770.

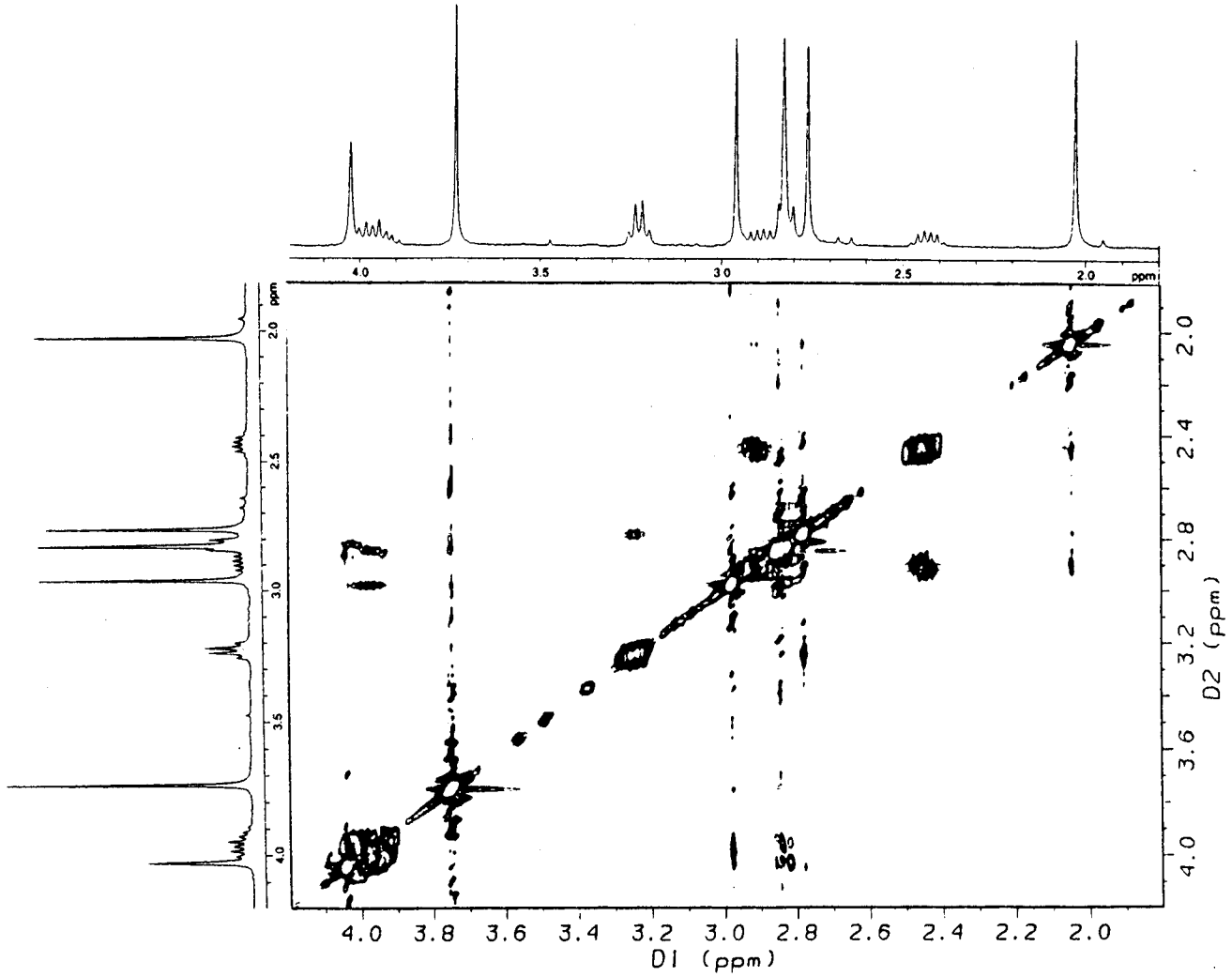


Figure 3. 2D ROSEY NMR spectrum of benzochlorin **15**.

base benzochlorins, and their Zn(II) analogues will be published elsewhere.

Photophysical Properties. The singlet oxygen ($^1O_2$) produced by a photosensitizer upon irradiation with light is believed to be the main cytotoxic agent for tumor necrosis in PDT treatment. Thus, the singlet oxygen quantum yields of two Zn(II) benzochlorins **11** and **21** were measured relative to tetraphenylporphine (TPP) in benzene by monitoring the temporal changes of the NIR luminescence intensities resulting from photoexcitation at 355 nm.[27] All samples yielded NIR luminescences showing a prompt increase in intensity which decayed and a slow decaying component which results from the luminscence of singlet oxygen.

Difference in triplet−triplet absorptions of the compounds were similar in both argon- and air-saturated solutions. The absorption and fluorescence maxima (Q-bands), fluorescence and singlet oxygen yields, and triplet lifetimes of benzochlorins **11** and **21** are summarized in Table 1. The visible spectra of benzochlorin **8** and its Zn(II) complex **11** are shown in Figure 4.

Chemical Reactivity by Frontier Orbital Theory. To examine the physicochemical basis for the observed site selectivity of benzochlorin derivatives, we performed semiempirical molecular orbital calculations for some of these compounds. Our specific interest was to obtain an understanding in why the specific sites (C_5 and C_{13^2}) were preferred for the formylation and oxidation over other potential sites. According to the Frontier Orbital theory,[28] the most reactive atom center is identified from the largest frontier orbital density or the largest frontier orbital coefficient.[29]

On the basis of the charge density obtained from Hückel π molecular orbital approximation, Pullman[30] showed the susceptibility of methine bridge carbons adjacent to the reduced pyrroline ring toward electro-

Table 1. Absorption and Fluorescence Maxima (Q-bands), Triplet Lifetime ($\tau_T/\mu s$), Fluorescence (Φ_f), and Singlet Oxygen Yields (Φ_Δ) of Benzochlorins 11 and 21

compound	absorption (λ_{max})	emission (λ_{max})	$\tau_T/\mu s$[a]	Φ_f[b]	Φ_Δ[c]
11	753 nm	771	190	0.03	0.50
21	672 nm	681	310	0.123	0.50

[a] Lifetime of the triplet state measured in argon-saturated benzene.[b] Fluorescence quantum yield referenced to meso-tetraphenylporphine in benzene for which $\Phi_f = 0.11$.[27] [c] Singlet oxygen quantum yield measured vis near-IR luminescence at 1270 nm, air-saturated benzene solvent; referenced to *meso*-TPP $\Phi_\Delta = 0.58$.[32b]

(27) Harriman, A. *J. Chem. Soc., Faraday Trans. 1* **1980**, *76*, 2256.

(28) (a) Fukui, K.; Yonezawa, T.; Shingu, H. *J. Chem. Phys.* **1952**, *20*, 722. (b) Fukui, K.; Yonezawa, T.; Nagata, C.; Shingu, H. *J. Chem. Phys.* **1954**, *22*, 1433. (c) Fukui, K. *Angew. Chem., Int. Ed. Engl.* **1982**, *21*, 801.

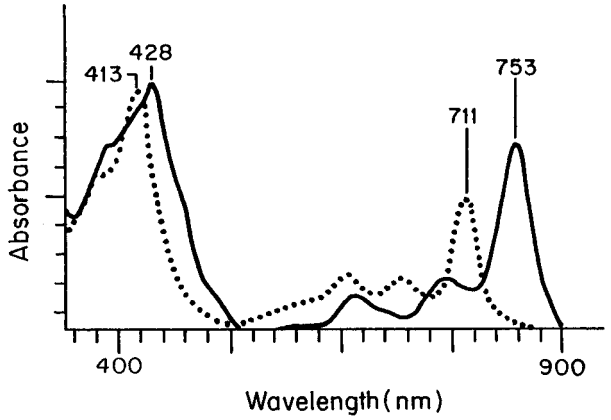
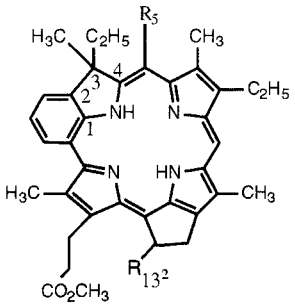


Figure 4. Electron absorption spectrum (in CH_2Cl_2) of benzochlorin **8** (---) and its Zn(II) analog **11** (—).

philic attack. This finding was confirmed later by another π molecular orbital approximation, PPP, by Fuhrhop and colleagues[31] who obtained a good correlation between their computational results and the valence bond theory based interpretation of the experimental results proposed by Woodward.[11] In most of the naturally occurring chlorin systems, two carbon atoms of the reduced ring exhibit sp^3 configurtion. In the benzochlorin system, on the other hand, only one of the carbon atoms (C3) of the reduced ring exists in a sp^3 configuration, while the other carbon atom (C2) belongs to a part of the benzene conjugated system. Thus, there may not be exact correlation between chlorin and benzochlorin systems. However, our results shown in Table 2 are in agreement with previous theoretical results on chlorins that the methine bridge carbons (C5, C20) adjacent to the reduced ring are more susceptible to the electrophilic attack than other methine carbons (C10, C15). This is true regardless of the nature of 13^2 substituents (2H or =O). The difference between these two compounds toward electrophilic attack is minimal while there is much more difference toward nucleophilic attack. The substitution at position C5 (R^5=H or formyl), has very little effect toward either nucleophilic or electrophilic attack. The examples of the frontier orbitals showing a large frontier orbital density around the C5 atom are shown in Figure 6, partsa and b.

Since the frontier orbital approach did not provide an insight into the difference in reactivity of the two carbons $C13^1$ and $C13^2$, we examined the net charges of the compound **7** and compound **14** as a free base by Mulliken population analyses. Mulliken charges of selected atoms for these compounds are shown in Table 3 where it is clearly shown that $C13^2$ has higher electron density than $C13^1$ for compound **7**. The trend remains the same after an introduction of a formyl group at C5 position. Thus, this is an indication that $C13^2$ may be more susceptible to electrophilic attack than $C13^1$ if electrostatic interaction is the dominant factor over others such as steric effects (accessibility) and solvents that are known to affect the reactivity of the molecules.

Table 2. Frontier Orbital Energy and Magnitude of Coefficients of Benzochlorin Derivatives



	HOMO-1	HOMO	LUMO	LUMO+1
when R_{13}^2 = O, R_5 = H:				
E (ev)	−8.192	−7.712	−1.760	−1.090
C5	0.39	0.23	0.05	0.10
C10	0.12	0.22	0.17	0.46
C15	0.18	0.07	0.42	0.17
C20	0.31	0.05 0.02	0.13	
when R_{13}^2 = 2H, R_5 = H				
E (ev)	−7.952	−7.551	−1.382	−0.835
C5	0.40	0.21	0.03	0.11
C10	0.15	0.21	0.30	0.39
C15	0.18	0.07	0.39	0.31
C20	0.31	0.03	0.01	0.15
when R_{13}^2 = O, R_5 = CHO[a]				
E (ev)	−8.390	−7.851	−1.872	−1.214
C5	0.43	0.22	0.05	0.10
C10	0.14	0.21	0.17	0.45
C15	0.18	0.08	0.42	0.17
C20	0.30	0.03 0.03	0.14	
when R_{13}^2 = 2H, R_5 = CHO[a]				
E (ev)	−8.177	−7.708	−1.501	−0.967
C5	0.44	0.19	0.04	0.12
C10	0.16	0.19	0.30	0.38
C15	0.17	0.08	0.39	0.29
C20	0.29	0.00 0.02	0.17	

[a] Although the formyl substituent can exist in two different conformations, only one of them is shown in this table since there was no substantial difference in the frontier orbital energy and coefficients for two conformers.

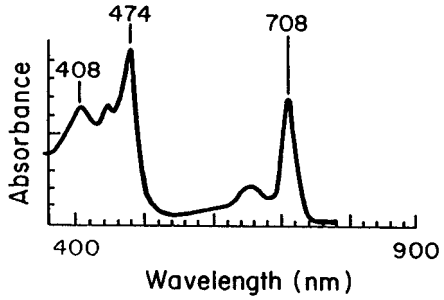


Figure 5. Electronic absorption spectrum (in CH_2Cl_2) of benzochlorin **13**.

Experimental Section

Melting points, which are uncorrected, were measured on a hot stage apparatus. Electronic absorption spectra were measured in dichloromethane solutions, and mass spectra were obtained at the Department of Biochemistry, Mass Spectrometry Facility, Michigan State University, East Lansing. The ^1H chemical shifts are reported relative to $CHCl_3$ at 7.26 ppm and ^{13}C shifts at 77.0 ppm. Reactions were monitored by thin-layer chromatography (TLC) by using cut strips (approximately 2 × 6 cm) of E. Merck silica gel 60 F254

(29) For example, (a) Sisti, N. J.; Motorina, I. A.; Dau, M.-E. T. H.; Riche, C.; Fowler, F. W.; Grierson, D. S. *J. Org. Chem.* **1996**, *61*, 3715. (b) Suzuki, H.; Tatsumi, A.; Ishibashi, T.; Mori, T. *J. Chem. Soc., Perkin Trans. 1* **1995**, 339. (c) Nicholls, A. W.; Akira, K.; Lindon, J. C.; Farrant, R. D.; Wilson, I. D.; Harding, J.; Killick, D. A.; Nicholson, J. K. *Chem. Res. Toxicol.* **1996**, *9*, 1414. (d) Boger, D. L.; Corbett, W. L.; Curran, T. T.; Kasper, A. M. *J. Am. Chem. Soc.* **1991**, *113*, 1713.

(30) Pullman, A. E. *J. Am. Chem. Soc.* **1963**, *85*, 366.

(31) (a) Fuhrhop, J.-H.; Subramanian, J. *Philos. Trans. R. Soc. London B* **1976**, *273*. (b) Fuhrhop, J.-H. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. II, Structure and Synthesis, Part B, p 131.

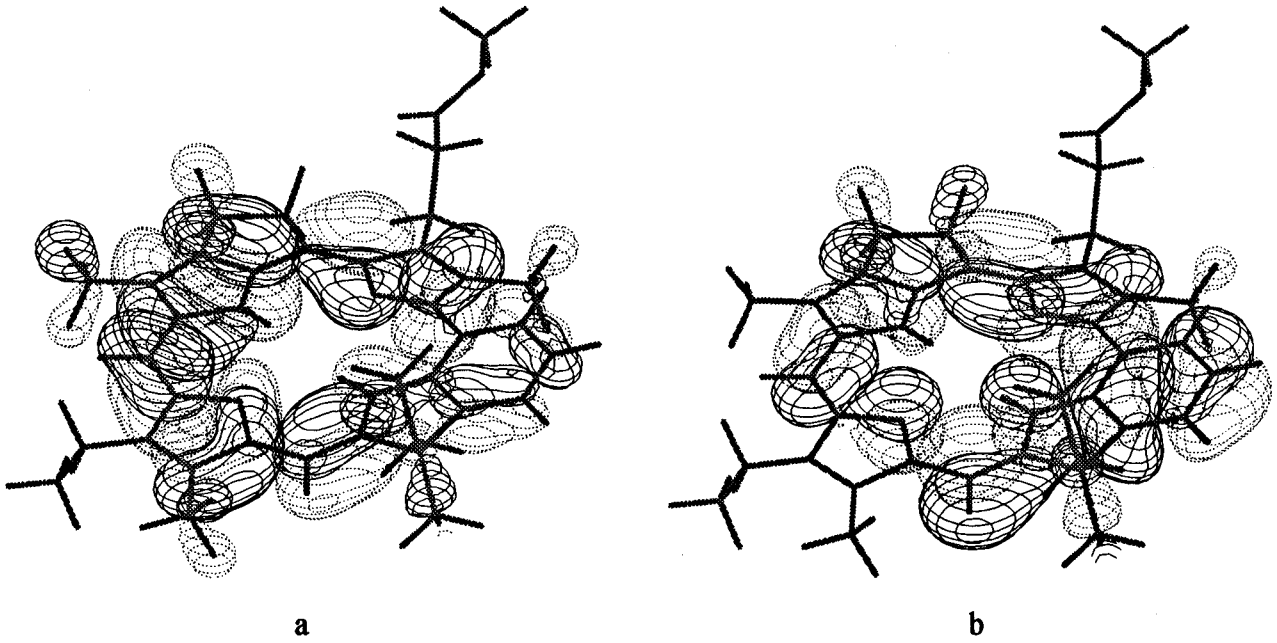
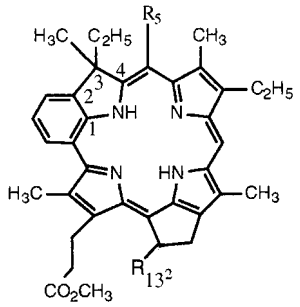


Figure 6. The frontier orbital contour map was drawn with MODEN program with the contour level of 0.03; a) HOMO of compound **7**, b) HOMO-1 of compound **7**.

Table 3. Mulliken Charges of Benzochlorin Derivatives with and without Formyl Substituent



	Mulliken Charges	
atom	$R_{13}^2 = 2H$, $R_5 = H$	$R_{13}^2 = 2H$, $R_5 = CHO$[a]
C5	−0.28	−0.29
C10	−0.07	−0.06
C15	0.09	0.09
C20	−0.07	−0.06
$C13^1$	−0.18	−0.18
$C13^2$	−0.24	−0.24

[a] Although the formyl substituent can exist in two different conformations, only one of them is shown in this table since there was no substantial difference in the Mulliken charges for two conformers.

precoated (0.25 mm thickness) plastic-backed sheets. Preparative TLC was performed on 20 × 20 cm TLC plates (Analtech). Two types of packing material were employed in column chromatography; E. Merck neutral alumina (70−230 mesh) and Merck silica gel 60. The alumina was deactivated with 6% H_2O (Brockmann Grade III) before use. Solvents were distilled prior to use.

Fluorescence quantum yields were measured on a "per photon basis", relative to tetraphenylporphyrin (TPP) in benzene. The ground-state absorbance of the sample solutions was matched at 0.05 (excitation wavelength: 410 nm). Fluorescence emission spectra of the reference solution and the sample solutions were recorded at room temperature; excitation and emission slits were set at 2.5 nm). The fluorescence spectrometer was corrected for wavelength distortions.[32]

Triplet−triplet absorption spectra and kinetics were obtained using a custom-built kinetic absorption spectrophotometer coupled to a Q-switched Nd:YAG laser for excitation. Singlet oxygen quantum yields were carried out using a time-resolved method.[32]

Molecular Modeling and Molecular Orbital Calculations. The crystal structure of the benzochlorin **8** was used as a starting geometry for the molecular modeling of other derivatives as well as the semiempirical molecular orbital geometry optimization. Molecular modeling program SYBYL (v6.03) was used to generate these compounds by performing appropriate substitutions and additions using standard geometry. All hydrogen atoms were generated by SYBYL molecular modeling program using standard geometry. Once the model was built, a partial molecular mechanics optimization was performed to relieve any constraints introduced by the modification of the crystal structure.

The geometry optimization of the compounds **7**, **8**, **14**, and **15** as a free base at ground state were performed with MOPAC 6.0 on a Sun Sparcstation2 at RHF AM1 level. Default geometry optimization procedure and criteria were used to obtain initial structures. Geometries were further refined with SCFCRT = 0.0000001 and GNORM = 0.1 by using the EF method. The frontier orbitals listed in Table 2 were obtained for these refined geometries at RHF-AM1 level. Some of these orbitals were shown in Figure 6, parts a and b (benzochlorin **7**), which were drawn by the program MOLDEN 3.2 installed on a Silicon Graphics INDIGO workstation.

Ni(II) Methyl 20-(2′-formylvinyl)-13^1-deoxypyropheophorbide-*a* 4. Phosphorus oxychloride (5 mL, 53.0 mmol) was added dropwise to a solution of 3-(dimethylamino)acrolein (5 mL, 4.9 mmol) in dichloromethane (10 mL), and the mixture was kept at 0 °C for 15 min. This mixture was then warmed to room temperature and added to a solution of Ni-*meso*-13^2-deoxypheophorbide-*a* **3** (1.0 g, 1.6 mmol) in 25 mL of dry dichloromethane in small amounts with continuous stirring over a period of 15 min. The final mixture was stirred at room temperature for 3 h. Saturated aqueous sodium carbonate was then added, and the solution was stirred overnight. The mixture was then extracted with dichloromethane, the combined organic layer was washed with water (3 × 200 mL) and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The resulting residue was chromatographed on silica gel (elution with 1% methanol in dichlo-

(32) (a) Zheng, X.; Rodgers, M. A. J. *J. Phys. Chem.* **1996**, *99*, 12797−12803. (b) Rossboich, et al. *J. Photochem.* **1985**, *31*, 37.

romethane), and the desired compound was collected and reprecipitated from dichloromethane/hexane to give 600 mg (55% yield) of the desired compound. Mp 298 °C. Vis in CH_2Cl_2 [λ_{max} (ϵ)]: 429 (103169); 509 (6587); 566 (11373); 654 (26227). ^{1}H NMR (δ ppm): 9.75 (d, CHO, J = 8.1 Hz,); 8.92 and 8.81 (s, 2 *meso* H); 8.51 (d, H of acrylaldehyde, J = 15.8 Hz), 5.82 (dd, (H of acrylaldehyde); 4.53 (m, 1H, 13b-CH_2); 4.32 (m, 1H, 18-H); 3.96 (m, 1H, 17-H); 3.84 (m, 1H, 13b-CH_2); 3.63 (s, 3 H, CO_2CH_3); 3.49 (overlapping q, 6 H, 2-CH_2 of Et and 13a-CH_2) 3.08, 3.06, and 2.84 (s, each 3H, 2, 6, 11-CH_3); 2.40 (m, 2H, 17-CH_2CH_2) 2.16 (m, 2H, 17-CH_2CH_2); 1.50 (m, 7H, 2-CH_3 of Et); 1.18 (d, J = 6.6 Hz, 3H, 18-CH_3). HRMS *m*/*z* calcd for Ni(II) $C_{37}H_{40}N_4O_3$: 646.2454, found 646.2432.

Ni(II) Benzochlorin 6. (Formylvinyl)-13^2-oxopyropheophorbide **4** (600 mg, 0.93 mmol) was stirred in TFA for 45 min. The reaction was monitored by following the shift in the Soret band by UV−visible spectroscopy. The mixture was poured into ice/water (300 mL), neutralized with 50% aqueous saturated sodium bicarbonate, and extracted with dichloromethane. The organic layers were washed with water (2 × 200 mL) and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The residue was chromatographed on silica gel (elution with dichloromethane). The desired compound was collected and recrystallized from dichloromethane/hexane to give 235 mg (40.28%) of the compound. Mp 295 °C. Vis in CH_2Cl_2 [λ_{max} (ϵ)]: 411 (59119); 495 (3333); 541 (4213); 603 (7547); 657 (29874). ^{1}H NMR (δ, ppm): 8.9 (d, 1 benzo H, J = 8.0 Hz,), 8.49 (s, 1 *meso* H), 7.8 (m, 2 benzo H and 1 *meso* H), 4.62 (m, 2H, 13b-CH_2), 3.86 (m, 2H, 17-CH_2-CH_2), 3.78 (s, 3H, CO_2CH_3), 3.60 (m, 2H, 13a-CH_2), 3.42 (q, 2H, 8-CH_2CH_3, J = 7.6 Hz), 3.18, 2.98, and 2.92 (s, 3H each, 7, 12, and 18 CH_3), 2.85 (m, 2H, 17-CH_2CH_2), 2.41 (m, 2H, 3-CH_2CH_3), 1.84 (s, 3H, 3-CH_3), 1.56 (t, 3H, 8-CH_2CH_3, J = 7.5 Hz,), 0.18 (t, 3H, 3-CH_2CH_3, J = 8.1 Hz). HRMS *m*/*z* calcd for $C_{37}H_{38}N_4O_2Ni$: 628.2348, found 628.2333

Benzochlorin 7. Ni(II) benzochlorin **6** (200 mg, 0.318 mmol) was dissolved in concentrated sulfuric acid (10 mL) and stirred at room temperature for 1 h. The resulting mixture was poured into ice/water (300 mL), neutralized with 50% aqueous saturated sodium bicarbonate, and extracted with dichloromethane. The organic layers were washed with water (3 × 100 mL) and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The residue was chromatographed on silica gel (elution with 2% CH_3OH−CH_2-Cl_2). The title compound was collected and recrystallized from dichloromethane/hexane to give 80 mg (44%). Mp 298 °C. Vis in CH_2Cl_2 [λ_{max} (ϵ)]: 400 (110000); 523 (11700); 592 (17000); 642 (34600). ^{1}H NMR (δ, ppm, $CDCl_3$): 9.38 (t, 1 benzo H, J = 5.1 Hz,), 8.28 (s, 1 *meso* H), 8.00 (m, 2 benzo H), 7.75 (s, 1 *meso* H), 4.79 (m, 2H, 13b-CH_2CH_2), 4.01 (m, 2H, 17-CH_2CH_2), 3.79 (s, 3H, CO_2CH_3), 3.6 (m, 2H, 13a-CH_2CH_2), 3.49 (q, 2H, 8-CH_2CH_3, J = 7.6 Hz), 3.32, 3.03, and 3.00 (s, 3H each, 7, 12, and 18-CH_3), 2.88 (m, 2H, 17-CH_2CH_2), 2.57 (m, 2H, 3-CH_2-CH_3), 1.87 (s, 3H, 3-CH_3), 1.62 (t, 3H, 8-CH_2CH_3, J = 7.5 Hz,), 0.10 (t, 3H, 3-CH_2CH_3, J = 7.4 Hz). HRMS *m*/*z* calcd for $C_{37}H_{40}N_4O_2$: 572.3132, found 572.3151.

13^2-Oxobenzochlorin 22 and 13^1-Methoxy-13^2-oxobenzochlorin (as a diasteriomeric mixture) 9. Free base benzochlorin **7** (145 mg, 0.253 mmol) was dissolved in 50 mL of methanol, and 190 mg (0.83 mmol) of DDQ was added. The mixture was refluxed for 2.45 h under nitrogen. A 150 mL volume of dichloromethane was added to the reaction mixture and washed with water (3 × 100 mL). The organic layer was dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. Purification of the residue by preparative TLC silica gel plates (2% methanol in dichloromethane) afforded free base ketobenzochlorin, compound **8** (faster moving, 60 mg, 40.3%), and free base ketomethoxybenzochlorin, compound **9** (slower moving, 42 mg, 26.8%).

13^2-Oxobenzochlorin 8. Mp 230 °C. Vis in CH_2Cl_2 [λ_{max} (ϵ)]: 411 (42756), 600 (9970), 651 (10263), 711 (21759). ^{1}H NMR in $CDCl_3$ (δ, ppm): 9.48 (d, 1 benzo H, J = 7.4 Hz), 8.41 (s, 1 *meso* H), 8.11 (m, 2 benzo H and 1 *meso* H), 4.20 (m, 4H, 17-CH_2CH_2 and 13a-CH_2), 3.77 (s, 3H, CO_2CH_3) 3.54 (q, 2H, 8-CH_2CH_3, J = 7.8 Hz), 3.32 (s, 3H, 18-CH_3), 3.12 (m, 5H, 7-CH_3 and 17-CH_2CH_2), 3.03 (s, 3H, 12-CH_3), 2.64 (m, 2H, 3-CH_2CH_3), 1.91 (s, 3H, 3-CH_3), 1.65 (t, 3H, 8-CH_2CH_3, J = 7.3 Hz), 0.114 (t, 3H, 3-CH_2CH_3, J = 7.5 Hz). HRMS *m*/*z* calcd for $C_{37}H_{38}N_4O_3$: 586.2944, found 586.2961.

13^1-Methoxy-13^2-oxobenzochlorin 9. Mp 262 °C. Vis in CH_2Cl_2 [λ_{max} (ϵ)]: 414 (47000), 606 (10300), 645 (10850), 708 (26200). ^{1}H NMR in $CDCl_3$ (δ, ppm): 9.49 (d, 1 benzo H, J = 6.4 Hz), 8.59 (s, 1 *meso* H, position 10), 8.19 (s, 1 *meso* H, position 5), 8.14 (m, 2 benzo H), 5.90 (s, 1H, 13a-H), 4.28 (m, 2H, 17-CH_2CH_2), 3.75 (s, 3H, CO_2CH_3), 3.71 (s, 3H, 13a-OCH_3), 3.59 (q, 2H, 8-CH_2CH_3, J = 7.7), 3.36 (s, 3H, 18-CH_3), 3.27 (m, 1H, 17-CH_2CH_2), 3.23 (s, 3H, 12-CH_3), 3.16 (s, 3H, 7-CH_3), 3.11 (m, 1H, 17-CH_2CH_2), 2.67 (m, 2H, 3-CH_2CH_3), 1.94 (s, 3H, 3-CH_3), 1.68 (t, 3H, 8-CH_2CH_3, J = 6.7 Hz), 0.075 (t, 3H, 3-CH_2-CH_3, J = 7.4). HRMS *m*/*z* calcd for $C_{38}H_{40}N_4O_4$: 616.3049, found 616.3050.

Zn(II) 13^2-Oxobenzochlorin 11. Free base ketobenzochlorin **8** (90 mg, 0.15 mmol) was dissolved in 50 mL of dichloromethane, and 60 mg of zinc acetate dissolved in 25 mL of methanol was added. The mixture was stirred for 90 min at room temperature. The reaction mixture was then washed with water (3 × 50 mL) and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The residue was chromatographed by preparative TLC silica gel plates (2% acetone in dichloromethane) to obtain 54 mg of the title compound. Mp 210 °C. Vis in CH_2Cl_2 [λ_{max} (ϵ)]: 429 (47021), 609 (9139), 693 (12448) 753 (37534). ^{1}H NMR in $CDCl_3$ (δ, ppm): 9.62 (d, 1 benzo H, J = 8.6 Hz), 8.44 (s, 1 *meso* H, position 10), 8.24 (s, 1 *meso* H, position 5), 8.19 (m, 1 benzo H), 8.15 (m, 1 benzo H), 4.13 (m, 1H, 17-CH_2CH_2), 4.00 (m, 1H, 17-CH_2CH_2), 3.91 (s, 2H, 13a-CH_2), 3.76 (s, 3H, CO_2-CH_3), 3.53 (q, 2H, 8-CH_2CH_3, J = 7.2 Hz), 3.32 (s, 3H, 18-CH_3), 3.14 (s, 3H, 7-CH_3), 2.94 (t, 2H, 17-CH_2CH_2, J = 7.9 Hz), 2.82 (s, 3H, 12-CH_3), 2.67 (m, 2H, 3-CH_2CH_3), 2.14 (s,3H, 3-CH_3), 1.62 (t, 3H, 8- CH_2CH_3, J = 9 Hz), 0.13 (t, 3H, 3-CH_2-CH_3, J = 8 Hz). HRMS, *m*/*z* calcd for $C_{37}H_{36}N_4O_3Zn$: 648.2079, found 648.2090.

Zn(II) 13^1-Methoxy-13^2-oxobenzochlorin 12. Free base ketomethoxybenzochlorin **9** (25 mg, 4.0 mol) was dissolved in 15 mL of dichloromethane, and 30 mg of zinc acetate dissolved in 10 mL of methanol was added. The mixture was stirred for 90 min at room temperature. The reaction mixture was then washed with water (3 × 50 mL) and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The residue was chromatographed by preparative TLC silica gel plates (2% acetone in dichloromethane) to obtain 15 mg (54.5%) of the title compound. Mp > 300 °C. Vis in CH_2Cl_2 [λ_{max} (ϵ)]: 423 (35620), 612 (7050), 705 (9900), 750 (21150). ^{1}H NMR in $CDCl_3$ (δ, ppm): 9.68 (m, 1 benzo H), 8.8 (d, 1 *meso* H), 8.29 (s, 1 *meso* H), 8.20 (m, 1 benzo H), 8.14 (m, 1 benzo H), 5.01 (s broad, 1H, 13a-H), 4.08 (m, 2H, 17-CH_2CH_2), 3.73 (d, 3H, CO_2CH_3), 3.68 (m, 2H, 17-CH_2CH_2), 3.43 (d, 3H, 13a-OCH_3), 3.16 (s, 3H, 18-CH_3), 3.07 (m, 3H, 12-CH_3), 2.74 (m, 7H, 7-CH_3, 3 and 8-CH_2CH_3), 2.12 (d, 3H, 3-CH_3), 1.73 (dt, 3H, 8-CH_2CH_3), 0.20 (dt, 3H, 3-CH_2CH_3). ^{13}C NMR (δ, ppm, total C = 37): 203.74, 174.39, 173.99, 157.59, 156.95, 154.06, 151.96, 149.83, 144.93, 143.21, 138.04, 138.88, 135.06, 130.38, 127.25, 125.66, 120.53, 118.00, 116.20, 116.04, 98.33, 89.89, 55.06, 51.34, 37.49, 36.29, 36.05, 28.44, 22.92, 19.16, 16.85, 16.62, 11.22, 10.91, 8.84. HRMS *m*/*z* calcd for $C_{37}H_{36}N_4O_3Zn$: 678.2184, found 678.2156.

13^1,13^2-Dioxobenzochlorin 13. Benzochlorin free base **9** (21 mg) was dissolved in dichloromethane (5 mL), and DDQ (26 mg) dissolved in ethanol (15 mL) was added to it. The reaction mixture was refluxed for 3 h and monitored spectrophotometrically. A shift in wavelength from 642 to 708 nm was observed (see Figure 5). The reaction mixture was cooled, diluted with dichloromethane (50 mL), and washed with water. The organic layer was separated and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was purified by preparative plates using 2% methanol in dichloromethane as eluent. The solvents were evaporated, and the compound was crystallized from CH_2Cl_2/hexane. Yield

6 mg. Mp 290 °C. Vis in CH_2Cl_2 [λ_{max} (ϵ)]: 474 (38700); 544 (8900); 708 (24620). ^{1}H NMR in $CDCl_3$ (δ, ppm) 9.66 (d, 1 benzo H, J = 8.5 Hz), 8.88 (s, *meso* H), 8.45 (s, *meso* H), 8.30 (m, 2 benzo H), 4.39 (m, 2H, 17-CH_2CH_2), 3.72 (s, 3H, CO_2CH_3), 3.68 (q, 2H, 8-CH_2CH_3), 3.50, 3.42 and 3.26 (s, each 3H, 7, 12, and 18-CH_3), 3.10 (t, 2H, 17-CH_2CH_2), 2.74 (m, 2H, 3-CH_2CH_3), 2.04 (s, 3H, 3-CH_3), 1.72 (t, 3H, 8-CH_2CH_3, J = 7.7 Hz), 0.091 (t, 3H, 3-CH_2CH_3, J = 7.3 Hz). HRMS *m/z* calculated for $C_{37}H_{36}N_4O_4$: 600.2736. Found: 600.2723.

5-Formyl-13^2-oxobenzochlorin 15. Phosphorus oxychloride (1 mL, 10.1 mmol) was added dropwise to a solution of DMF (1 mL, 12.8 mmol) in dichloroethane (10 mL), and the mixture was kept at 0 °C for 15 min. This mixture was then warmed to room temperature and added to a solution of Ni-benzochlorin **6** (200 mg, 0.3 mmol) in 10 mL of dry dichloroethane in small amounts with continuous stirring over a period of 15 min. The final mixture refluxed under N_2 for 3 h. Saturated aqueous sodium carbonate (100 mL) was then added, and the solution was stirred overnight. The mixture was then extracted with dichloromethane and dried over anhydrous sodium sulfate, and the solvent was removed under vacuum. The resulting residue was chromatographed on silica gel (elution with 2% methanol in dichloromethane), and the desired compound was collected and recrystallized from dichloromethane/hexane to give 70 mg of the title compound. Mp 300 °C. Vis in CH_2Cl_2 [λ_{max} (ϵ)]: 432 (32340); 627 (5800); 702 (11000); 768 (31200). ^{1}H NMR in $CDCl_3$ (δ, ppm) 11.05 (s, C*H*O), 8.62 (d, 1 benzo H), 8.29 (s, 1 *meso* H), 7.72 (m, 2 benzo H), 4.04 (s, 2H, 13a), 3.96 (m, 2H, 17-CH_2CH_2), 3.74 (s, 3H, CO_2CH_3), 3.24 (q, 2H, J = 8 Hz), 2.97 (s, 3H, 18-CH_3), 2.84 (m, 6H, 17-CH_2CH_2, CH_3 on ring C, 3-CH_2CH_3), 2.77 (s, 3H, 7-CH_3) 2.44 (m, 1H, 3-CH_2CH_3), 2.04 (s, 3H, 3-CH_3), 1.46 (t, 3H, 8-CH_2CH_3, J = 7.7 Hz), 0.17 (t, 3H, 3-CH_2CH_3, J, = 6.9 Hz). C NMR (ppm, total C = 38): 202.42, 187.14, 173.91, 169.72, 152.71, 152.24, 149.51, 147.72, 146.22, 142.55, 141.71, 140.46, 138.50, 136.49, 133.65, 130.00, 127.23, 124.93, 121.35, 119.09, 118.89, 113.76, 104.14, 103.19, 57.21, 51.63, 38.16, 36.23, 35.69, 28.30, 22.76, 19.20, 16.32, 15.23, 15.16, 11.03, 9.06. HRMS *m/z* calcd for $C_{38}H_{36}N_4O_4Ni$: 614.2893, found 614.2876.

Crystal Structure Determination.[33] The techniques and programs used for crystallographic studies are similar to those reported by Senge and Smith.[12] Benzochlorin **8**, $C_{37}H_{38}N_4O_3$, at 130 K (Cu Kα radiation, λ = 1.54178 Å, $2q_{max}$ = 114°), monoclinic, space group $P2_1$, a = 13.384(7), b = 15.494(5), c = 15.840(6) Å, b = 114.41(4)°, V = 2991(2), Å^3, Z = 4, two crystallographically independent molecules refined against [F^2], 4135 independent reflections, 634 parameters, $R1$ = 0.0699 {$I > 2_\sigma(I)$, RI = 0.1135 (all data), $wR2$ = 0.1975 (all data).

Acknowledgment. This work was supported from the research grants funded by Mallinckrodt Medical Inc., St. Louis, the National Institutes of Health (CA 55791, HL 22252), partial support of the NMR facility by RPCI center support grant (CA 16056), the Oncologic Foundation of Buffalo, Deutsche Forschungemeinschaft (Heisenberg-Stipendium for M.O.S.), and the Fonds der Chemischen Industrie. We thank Dr. Sapna Gupta for the initial preparation of some benzochlorins and Professor M. A. J. Rodgers, Chemistry Department, Bowling Green State University, for photophysical characterization of Zn(II) benzochlorins. We express our appreciation to Dr. B. Paul and Mr. B. F. Mayer, Department of Experimental Therapeutics (core grant CA 16050), for isolating methylpheophorbide-*a* from *Spirulina pacifica*.

Supporting Information Available: X-ray data of benzochlorin **8** (13 pages). This material is obtained in libraries on microfiche, immediately follows this article in the microfilm version of this journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO971968P

(33) The authors have deposited atomic coordinates and a full structure description for benzochlorin **8** with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge on application to The Director, CCDC, 12 Union Road, Cambridge, CB2, 1EZ, UK (fax: int code +(1223) 336−033, e-mail: teched@chemcrys.cam.ac.uk).