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CHLOROPHYLL-A DERIVATIVES IN PHOTODYNAMIC THERAPY: EFFECT OF POSITION OF HEPTYL ETHER SIDE-CHAINS ON *IN VIVO* PHOTOSENSITIZING ACTIVITY

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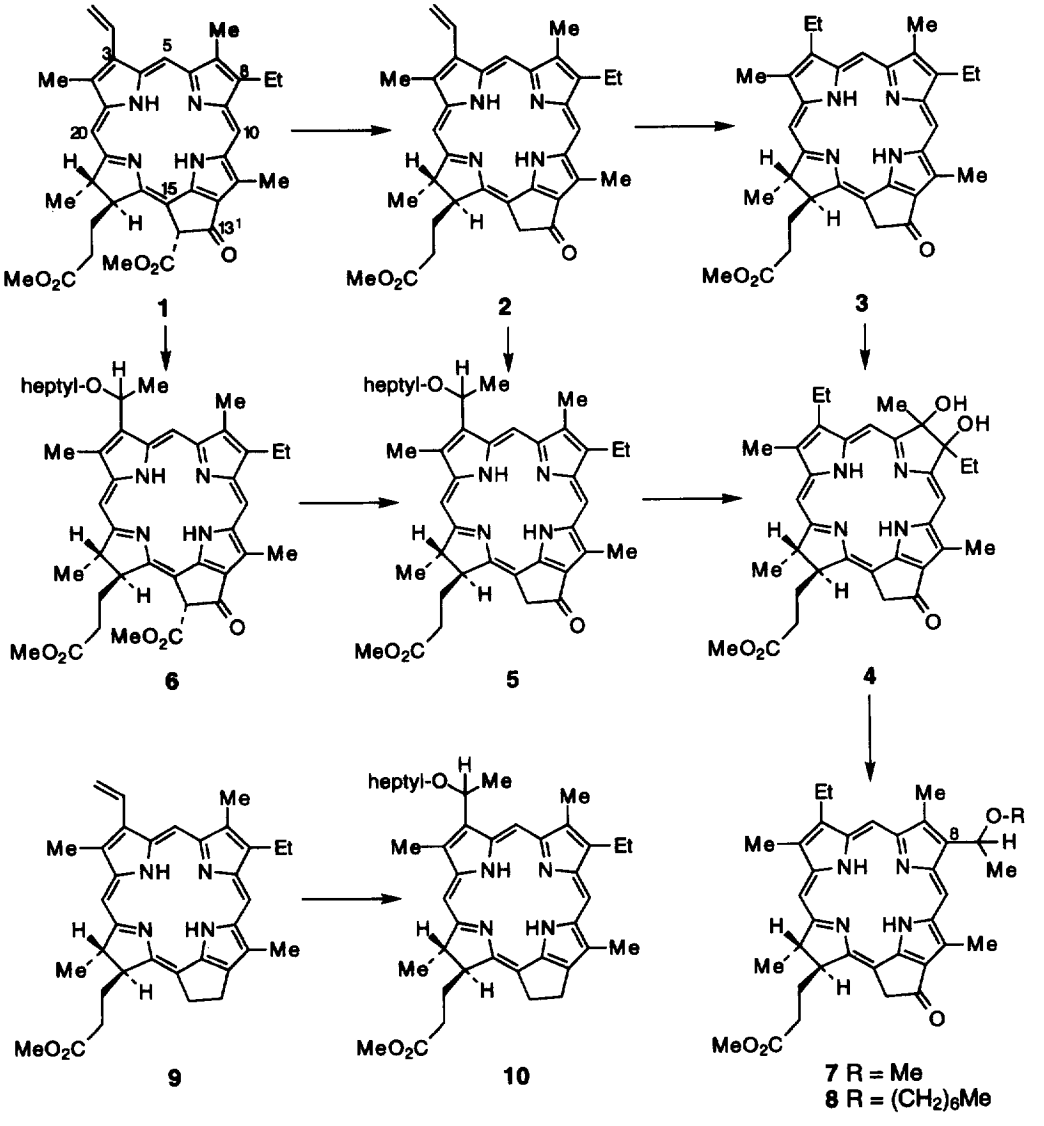
Abstract: Syntheses and comparative *in vivo* photosensitizing efficacy of a series of chlorophyll-a derivatives with (1-heptyloxyethyl) substituents at various positions are discussed. Compared with Photofrin®, the n-heptyl ether analogues showed better *in vivo* sensitizing activity (DBA/2 mice transplanted with SMT/F tumors) as well as reduced skin phototoxicity. The Vilsmeier formylation of copper(II) methyl mesopyropheophorbide-a **11** did not produce the expected meso-formyl derivative **12**, but instead gave the α,β-unsaturated chloroformyl analogue **13** as the major product.

Photodynamic therapy (PDT) has been shown to be effective against a variety of malignant tumors.[1] For *in vivo* application of PDT, preference is usually given to those sensitizers that exhibit absorption bands in the 600 to 850 nm region where the penetration of light into mammalian tissues is better. For a sensitizer to be effective, it should show preferential localization in tumors and have the ability to produce singlet oxygen ($^1O_2$) to facilitate tumor destruction. In recent years several porphyrin-type long-wavelength absorbing photosensitizers have been reported. However, there are only a few reports in which the effect(s) of various substituents upon a particular class of compounds have been studied.[2]

A few years ago, Moan and coworkers[3] prepared a series of alkyl ether analogues of hematoporphyrin derivatives and showed that an increase in the length of the ether carbon chain was accompanied by an increase in sensitizing activity. We later extended this study to the pheophorbide-a series and obtained similar results.[4] Storage (in 1% aqueous Tween 80) at room temperature caused the alkyl ether analogues of pheophorbide-a to be converted into the related pyropheophorbide-a analogues in which the methyl ester group attached to isocyclic ring was cleaved. We therefore focused our attention on the alkyl ether derivatives (1-12 carbon chain length) of pyropheophorbide-a. Among such derivatives, 2-(1-heptyloxyethyl)-pyropheophorbide-a was found to be extremely effective.[5]

In our attempts to investigate the effect of substituent regiochemistry on PDT activity, we initially introduced the heptyl ether side chain at the meso (20) position of methyl 13[1]-deoxypyropheophorbide-a.[6] Surprisingly, this compound was found to be less effective than the related pyropheophorbide analogue in which the heptyl ether group was present at the peripheral position of the tetrapyrrole unit. We have now modified this molecule further, and the first part of this report deals with an efficient approach for the preparation of alkyl ether analogues of pyropheophorbide-a in which the methyl- and heptyl-ether groups were introduced at position-8 of the macrocycle. For the preparation of these derivatives, methyl pheophorbide-a **1** was isolated from *Spirulina pacifica*.[7] It was then converted into methyl pyropheophorbide-a **2** and methyl mesopyropheophorbide-a **3** by following standard chemistry briefly depicted in

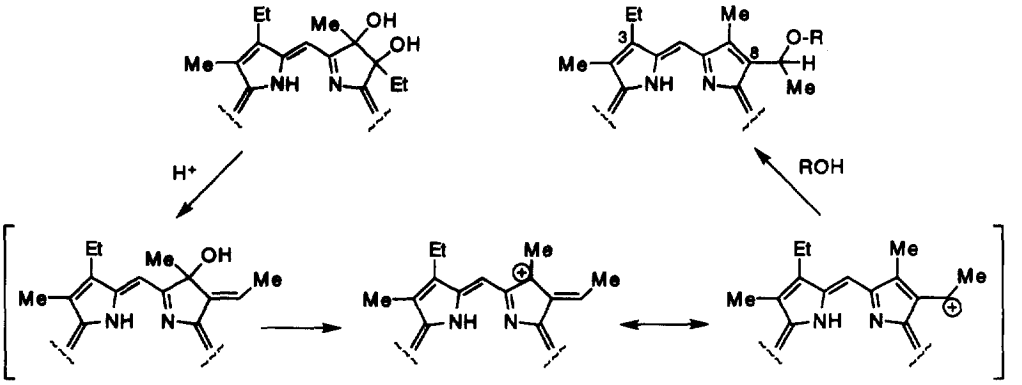
Scheme 1. Reaction of **3** with OsO_4/H_2S[8] gave the *vic*- dihydroxybacteriochlorin **4** (λ_{max} 711 nm), in 65% yield. Treatment of **4** with a few drops of HCl in refluxing methanol or 1-hexanol gave the corresponding alkyl ether analogues **7** and **8** in 50% and 45% yield, respectively.



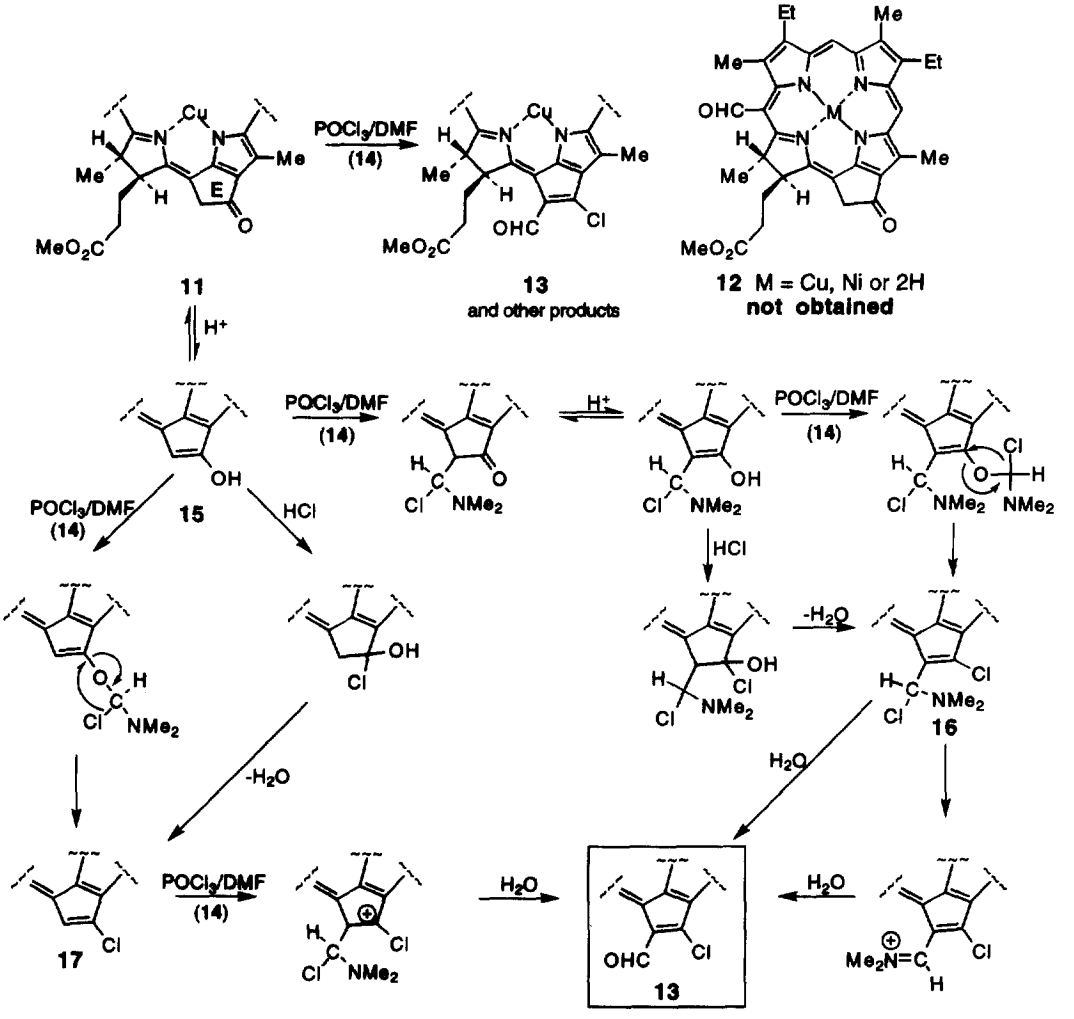
Scheme 1

The alkyl ether derivatives **7** and **8** have long-wavelength absorption maxima at 657 nm. The mechanism of the formation of alkyl ether analogue is shown in Scheme 2.

In order to shift the long-wavelength absorption further towards the red region, we made several attempts to introduce a formyl group at the 20–position of methyl mesopyropheophorbide-a **3**. Vilsmeier formylation of copper(II) methyl mesopyropheophorbide-a **11** according to the literature procedure,[9] however, did not give the desired 20-formylchlorin **12**, but instead gave the α,β-unsaturated chloroaldehyde **13**. There exists considerable literature precedent



Scheme 2: Proposed mechanism for formation of ethers from porphyrin vicinal-diols

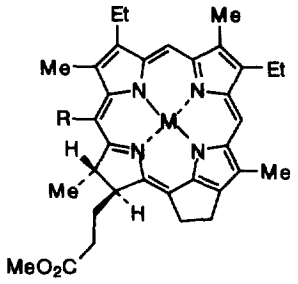


Scheme 3: Possible mechanisms for the formation of α,β-unsaturated chloroaldehyde **13**

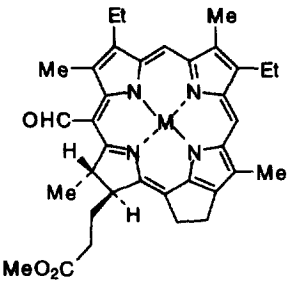
for the reaction of the Vilsmeier reagent with enolizable ketones.[10] Under the reaction conditions one can imagine enolization catalyzed by HCl to give **15**, followed by reaction with the electrophilic Vilsmeier reagent [$(Me_2N{=}CHCl)^+$], **14**. A second enolization, followed by the conversion of enol to vinyl chloride **16** and then hydrolysis will produce the α,β-unsaturated chloroaldehyde **13**. Alternatively, chlorination of **15** to give **17** followed by formylation of the double bond would give the observed product **13**. Such reactions in the chlorophyll derivative field have already been described.[11] Protection of the carbonyl function to prevent this unwanted side reaction was next considered. Protection as the ethylene ketal can be achieved by reacting **11** with ethylene glycol and trimethylsilylchloride.[12] However, the stability of the ethylenedioxy group to the acidic conditions of the Vilsmeier reaction was questionable. Finally, there are several reports dealing with reactivity of cyclic acetals and ketals toward the Vilsmeier reagent.[13]

The failure of the Vilsmeier formylation of mesopyropheophorbide-a **11** led us to consider the *in vivo* sensitizing effect of the 20–meso formyl group in the 13^1-deoxy- series (obtained by reaction of **3** with TFA/$NaBH_4$), and to compare the sensitizing efficacy of sensitizers **10** and **19**. As expected, Vilsmeier formylation of nickel(II) methyl 13^1-deoxypyropheophorbide-a **18** gave the desired 20-meso formyl analogue **20** in 75% yield.[14]

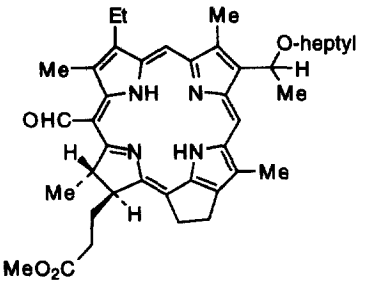
Removal of nickel from **20** with H_2SO_4 and reaction of the intermediate **21** with OsO_4/H_2S produced the related *vic*- dihydroxybacteriochlorin in 60% yield. This was then converted into methyl 8-deethyl-8-(1-heptyloxyethyl)-13^1-deoxypyropheophorbide-a **22** in 40% yield by following the procedure discussed for the synthesis of **8**.



18 M = Ni, R = H
19 M = 2H, R = CH_2O-heptyl



20 M = Ni
21 M = 2H



22

The absorption and fluorescence spectra of the newly synthesized compounds were measured in dichloromethane. Heptyl ether analogue **8** had a long-wavelength absorption at 657 nm. The related formyl analogue **22** showed a maximum at 672 nm. In sensitizer **8**, excitation of the peak at 657 nm gave strong fluorescence emission at 663 nm. Similar excitation of the peak at 672 nm in the formyl analogue **22** produced only about one sixth of the fluorescence intensity at 691 nm, indicating that in 13^1-deoxypyropheophorbides, introduction of electron-withdrawing groups such as formyl lead to a decrease in the fluorescence quantum yield.

Anti-tumor activity:

The *in vivo* photosensitizing activities of compounds **7**, **8**, **19** and **22** were compared with 3-(1-heptyloxyethyl)-analogues of methyl pyropheophorbide-a **5** and methyl 13^1-deoxypyropheophorbide-a **10** in DBA/2 mice bearing subcutaneously implanted SMT-F tumors, a method previously described by Dougherty *et al.*[15] The new sensitizers were first dissolved in a minimum quantity of Tween 80 and then diluted with distilled water to a final concentration of 1% Tween 80. Ten mice/group were used for our study. All the sensitizers were injected at a dose of 0.47μmol/kg, and the mice were treated 24 h post injection of the drug. Treatment conditions involved the use of a uniform light dose of

135 J/cm^2 (75 mW/cm^2) at the following wavelengths:[16] 665 nm for **5**; 660 nm for **7**; 660 nm for **8**; 660 nm for **10**; 660 nm for **19**; and 677 nm for **22**. The comparative *in vivo* sensitizing efficacy of various sensitizers at day 30 are shown in Figure 1.

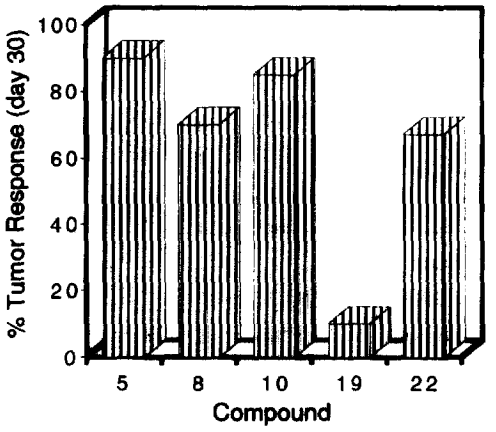


Figure 1: Comparative *in vivo* sensitizing effectiveness of sensitizers **5**, **8**, **10**, **19**, and **22** at day 30.

The 3-(1-heptyloxyethyl)pyropheophorbide-a **5** (90% tumor response) and related 13^1-deoxy analogue **10** were found to be quite effective. Under similar treatment conditions, methyl 8-(1-methoxyethyl)-mesopyropheophorbide-a **7** did not show any activity. Replacement of the methyl- with a heptyl- ether provided a remarkable improvement in anti-tumor activity, and compared with pyropheophorbide-a **5** (in which the heptyl ether group is present at position-3), sensitizer **8** was slightly less effective (70% tumor response). In the 13^1-deoxy series, the introduction of the formyl group in **22** gave a red shift of 22 nm with 67% tumor response at day 30, and was less effective than **10**. This study thus reveals that in contrast to sensitizer **19** in which (1-heptyloxyethyl) was substituted at the meso position, substitutions at positions 3- or 8- (in sensitizers **5**, **8**, **10** and **22**) did not make much difference in photosensitizing efficacy. Detailed mechanistic studies with these compounds are currently in progress.

Skin Phototoxicity (Foot Response Experiment):

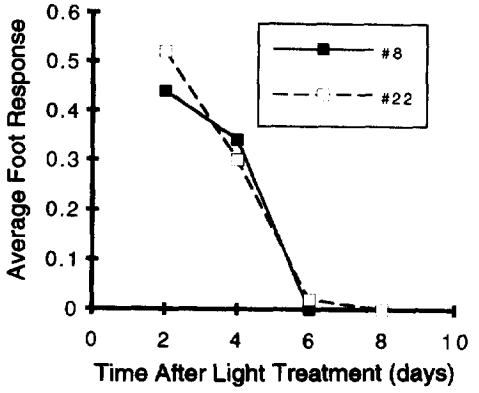


Figure 2: Average foot responses for sensitizers **8** and **22** after light treatment (see text).

Since prolonged cutaneous photo sensitivity is a potentially serious side effect of Photofrin® administration, we tested the phototoxicity of heptyl ether analogues **8** and **22**. In this experiment, five Swiss mice/group were used, and were given the doses (0.47 μmol/kg) that were used to determine the photosensitizing efficacy. After day 1, the mice were restrained without anesthesia in aluminum holders and one hind foot of each mouse was illuminated with light from a dye laser. Light dose (75 mW/cm^2, 135 J/cm^2) was measured with a Coherent 210 power meter and wavelength was measured with a PTR Optics monochromator. Foot response was graded as follows: Scale: 0, no difference from normal; 0.1, very slight edema; 0.3, slight edema; 0.5, moderate edema; 0.75, large edema or slight reddening. The average foot responses are shown in Figure 2. Here it can be seen that compounds **8** and **22** induced a minimal phototoxic response. Photofrin® at an equivalent dose induces reactions in excess of 1.5 (slight damage to toes).[4] Animals were sacrificed immediately after peak foot scores were obtained.

Acknowledgments:

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