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Efficient Synthesis of Pyropheophorbide-*a* and Its Derivatives

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Abstract:

A new and simplified method of preparing hexyloxy pyropheophorbide-*a* (HPPH), a promising agent used in photodynamic therapy, is described. This method is carried out in two processing steps, replacing an older method requiring five steps. This is accomplished by means of a Dieckmann condensation and subsequent thermal decarboxylation, both occurring in the same high-boiling solvent, thus reversing the long-standing trend in which naturally occurring chlorin derivatives with exocyclic rings are often subjected to conditions that open this ring to obtain chlorin derivatives. In the new process, a raw material without an exocyclic ring is used to construct a product containing the exocyclic ring. The new method does not require cryogenic processing or chromatography, removing the most significant obstacles to large-scale preparation of HPPH and its homologues.

Introduction

Photodynamic therapy (PDT)[1] is a relatively new treatment method for the destruction of tumors, hyperproliferative tissue, or other undesired structures. PDT is based on the accumulation of a photosensitizer in malignant tissue after administration. Subsequent illumination with light of an appropriate wavelength brings about a photochemical reaction, sometimes called a *photodynamic effect* (photochemical reaction producing singlet oxygen, $^1O_2$) that results in destruction of tumor or other tissue. It is well established[2] that both absorption and scattering of light by tissue increases as the wavelength decreases and that the most effective sensitizers are those that have strong absorption bands between 660 and 700 nm.

Historically, the usefulness of photosensitizers approved for human use, such as porfimer sodium (Photofrin), has been limited by certain properties, two of which are a less than ideal capacity to absorb light at useful wavelengths and persistence in the skin causing an undesirable and prolonged photosensitivity. This often requires that patients be protected from light for periods of several weeks after treatment. For these reasons, compounds with improved biological and photophysical properties have been sought. One such compound, hexyloxy pyropheophorbide-*a* (**4**), known as HPPH (Photochlor), offers increased wavelength of maximum absorbance and a greater molar extinction coefficient, improving the depth of light penetration for activation of the photosensitizer and efficient singlet oxygen generation in tumor tissue of both animal[3] and human subjects.[4] Compound **4** is a member of a homologous series of ethers originally derived[3] from pyropheophorbide-*a* (**6**) by acid-catalyzed hydration of the A-ring vinyl group in the presence of the appropriate alcohol. It was found that the choice of this alkyl group,[5] and the lipophilicity conferred on the molecule thereby, is critical in determining the in vivo activity[6] of the molecule and is responsible for the hexyl ether having the maximum activity of the members of this homologous series.

Previously, preparation of pyropheophorbide derivatives, including **4**, has involved a cumbersome extraction procedure nearly impossible to reproduce on the larger scale we expect will be required to support clinical or commercial use of this product. Herein we describe a new two-pot process in which those features of the original process that would be difficult to carry out on a larger scale have been removed, thus making it suitable for the preparation of larger amounts of **4**.

Discussion

The original synthetic process,[7] shown in Figure 1, required the isolation of methyl pheophorbide-*a* (**1**) from *Spirulina* algae by cryogenic fracturing of the cells with liquid nitrogen, followed by extraction, chromatographic purification, and recrystallization. The methyl pheophorbide-*a* **1** so obtained was then thermally decarboxylated in *sym*-collidine (2,4,6-trimethylpyridine) at reflux (about 175 °C). The resulting methyl pyropheophorbide-*a* (**2**) was treated with hexyl alcohol and acid to form the hexyl ether **3**. Finally, the methyl ester was removed by saponification to give HPPH **4**. Thus, a rather laborious natural product isolation, followed by three separate chemical steps was required to obtain HPPH **4**. This

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(6) 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*, 4000−4007.

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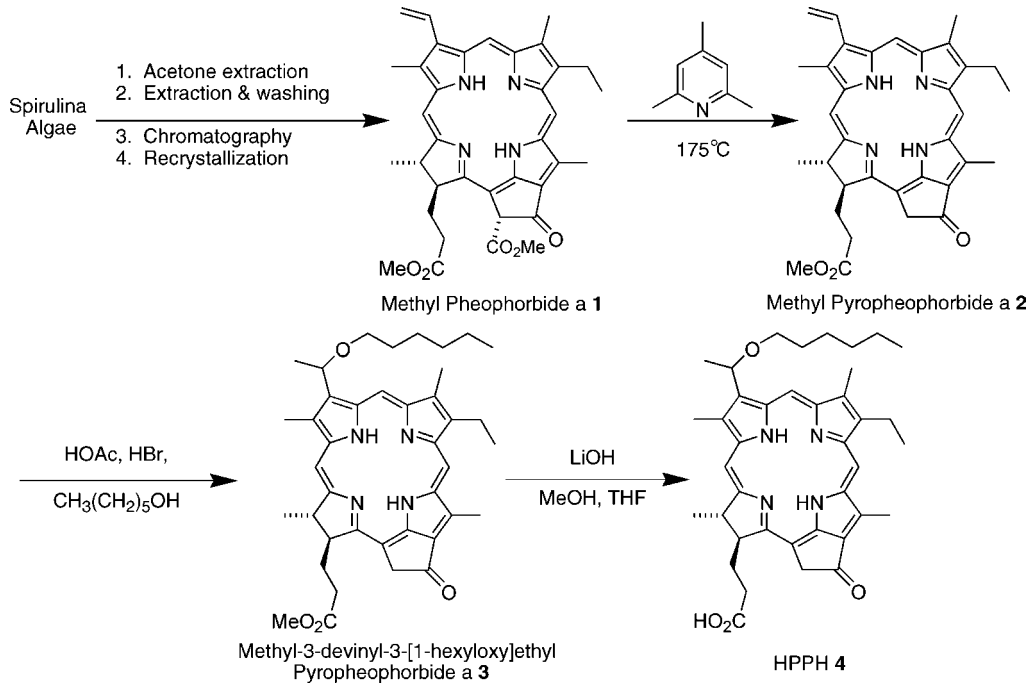

Figure 1. **Synthesis of HPPH 4 from methyl pheophorbide a (1).**

procedure works well in a laboratory-scale preparation where the final product is required in small amounts (milligrams). However, the purification of the intermediates at several stages of the synthesis required column chromatography. Therefore, for a large-scale preparation (roughly speaking, more than 100 mg), we were interested in developing an alternate route to prepare the desired compounds without the use of tedious purification techniques.

From our efforts on an unrelated project, we discovered that chlorin e_6 and its trimethyl ester **5** are considerably easier to obtain[8] than most pheophorbide-*a* derivatives. Inspection of the structure of **5** reveals that it appears to be an ideal substrate for an ester condensation reaction giving the pheophorbide nucleus as its product. Further examination of **5**, in three-dimensional form, reveals that it possesses almost ideal conformation for a condensation reaction between the α-carbon of the C-15 acetic acid side chain and the C-13 carboxyl carbon. Very reliable predictions of reactivity can usually be made using the set of criteria for ring-closure reactions known formally as Baldwin's Rules.[9] These rules suggest that this reaction, formally 5-*exo*-trig in configuration, should be kinetically favored (relatively rapid). Our investigation revealed that, in fact, chlorin e_6 trimethyl ester **5**, readily undergoes a Dieckmann condensation,[10] as shown in Figure 2, to give **1** as the principal product. This product has the additional, or exocyclic, ring[11] sometimes called an "E-ring" that is present in the pheophorbides and in chlorophyll itself.

Our application of the Dieckmann condensation was undertaken for the purpose of preparing pyropheophorbide-*a*

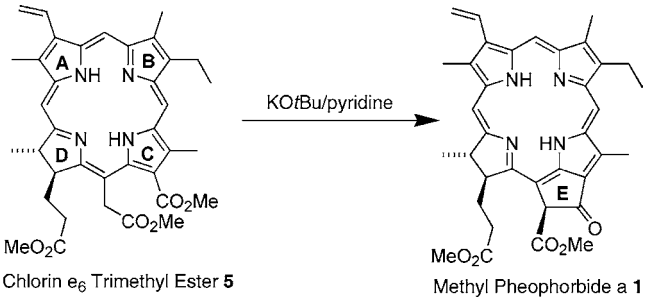

Figure 2. **Dieckmann condensation giving pheophorbide-*a* (1) from chlorin e_6 trimethyl ester 5.**

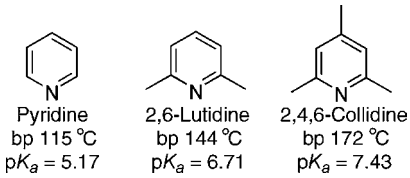

Figure 3. **Basic aromatic solvents in order of increasing basicity.**

derivatives such as **4**. For this reason, we were interested in the possibility of carrying out the subsequent decarboxylation, as in the conversion of **1** to **2** in Figure 1, in the same reaction mixture. Ideally, this would be done without isolation of the product of the condensation, presumably **1**, simply by raising the temperature to a sufficient level. The Dieckmann condensation has traditionally been performed in aromatic solvents, originally benzene, but later toluene and others for safety reasons. In other examples, basic aromatic solvents such as pyridine have been used for this purpose. Since the decarboxylation, when performed in discrete fashion as shown in Figure 1, is already performed

(8) Chlorin e_6 is commercially available from several sources, including Frontier Scientific Discovery Chemicals, P.O. Box 31, Logan, UT 84323-0031; URL: http://www.frontiersci.com/all.htm.

(9) Baldwin, J. E.; Lusch, M. J. *Tetrahedron* **1982**, *38*, 2939−2947.

(10) For a fully detailed review, see: (a) Schaefer, J. P.; Bloomfield, J. J. *Org. React.* **1967**, *15*, 1−203. (b) Davis, B. R.; Garrett, P. J. *Compr. Org. Syn.* **1991**, *2*, 806−829.

(11) Some precedent for the construction of the exocyclic ring can be found in the literature, for example see: Smith, K. M.; Bisset, G. M. F.; Bushell, M. J. *J. Org. Chem.* **1980**, *45*, 2218−2224, but none for the chlorin e_6 system.

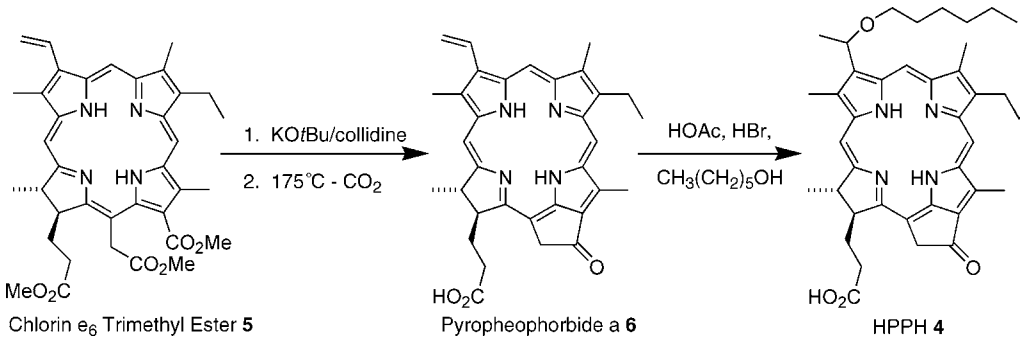

Figure 4. **Two-pot preparation of HPPH 4 from chlorin e_6 trimethyl ester 5.**

in a pyridine-type (π-deficient aromatic) base chosen especially for its high boiling point, we reasoned that this more substituted analogue of pyridine might be ideally suited for the Dieckmann condensation as well.

Although alkyl substitution of pyridine was initially considered in order to raise the boiling point of the reaction mixture, it also results in increased basicity and diminished nucleophilicity, two properties likely to enhance its usefulness in the Dieckmann condensation. Boiling points and pK_a values[12] for the principal methyl-substituted pyridines are shown in Figure 3. By employing these methyl-substituted pyridine homologues , the temperature of reflux of the reaction mixture is raised—the boiling point of pyridine is 115 °C, while that of *sym*-collidine is 172 °C—basicity is improved, and unwanted nucleophilicity at the nitrogen atom is minimized by steric hindrance. Thus, by raising the temperature of the reaction mixture after completion of the Dieckmann condensation, it is possible to bring about the subsequent thermal decarboxylation without any intervening purification or unnecessary manipulation of the reaction mixture.

As a further benefit, it was found that under the strongly basic conditions used for the Dieckmann condensation, the methyl ester of the pheophorbide system cleanly undergoes saponification, giving pyropheophorbide-*a* (**6**) itself, thus accomplishing what had been three separate chemical transformations in a single treatment, as shown in Figure 4. At this point, **6** need only be converted to its hexyl ether to produce HPPH **4**. This can be done according to the method used previously for this purpose,[13] giving an overall two-pot preparation of this product and the other members of its homologous series of alkyl ethers.

Conclusions

As part of our overall efforts to promote the viability of HPPH as a pharmaceutical product, it is necessary to demonstrate a synthetic pathway by which one can reasonably expect to produce the compound in useful amounts. The major impediments to the manufacture of HPPH on a scale sufficient to support clinical and commercial use of the compound in photodynamic therapy have been circumvented by the development of a new process reducing the number of processing steps from five to two and also eliminating the requirements for large-volume cryogenic processing and chromatographic purification. Studies designed to exploit the general utility of this new process for production of other photosensitizers are ongoing.

Experimental Section

NMR spectra were recorded in $CDCl_3$ solutions at 400 or 600 MHz on Bruker instruments. Chemical shifts are expressed in ppm with residual $CHCl_3$ in $CDCl_3$ (for 1H, 7.26 ppm) as internal standard. UV−vis spectra were recorded on a Varian Cary-50 Bio spectrophotometer. Spectral data were in agreement with published data.[13]

3-Devinyl-3-(1′-hexyloxy)ethyl-pyropheophorbide a (HPPH, 4.) Pyropheophorbide-*a* (**6**) (100 mg, 0.187 mmol) was taken in a 50-mL round-bottom flask, and 30% HBr/HOAc (Aldrich, 2.0 mL) was added. The reaction mixture was stirred at room temperature for 2 h, and the solvent was removed under high vacuum (bath temperature was maintained at 30−40 °C). The resulting concentrate was redissolved in dry dichloromethane (10 mL). Hexanol (2.00 mL) and potassium carbonate (200 mg) were added, and the reaction mixture was stirred at room temperature for 45 min under nitrogen atmosphere, poured into water (100 mL), and extracted with dichloromethane (3 × 100 mL). The organic layer was washed with water (2 × 200 mL) and dried over anhydrous sodium sulfate (5 g). Evaporation of the solvent at 30 °C and reduced pressure (ca. 20 Torr) gave a solid residue. This residue was crystallized by dissolving in a minimum amount of dichloromethane (10 mL), slowly adding hexanes (50 mL), and allowing the reaction mixture to stand overnight at about 5 °C to give 84 mg (71%) of **4**; ^{1}H NMR ($CDCl_3$, δ ppm): 9.77 and 9.52 8.50 (s, 1H, *meso*-H); 5.90 [q, 1H, C*H*(o-hexyl)-CH_3); 5.22 (dd, 2H, 2H, exocyclic ring); 4.41 (q, 1H, 18H); 4.28 (d, 1H, 17H); 3.75 (q, 2H, CH_2CH_3); 3.62, 3.25 and 3.20 (each s, 3H, ring CH_3); 2.10 (3H, CHCH_3); 1.80 (d, 3H, 18-CH_3): 1.75 (t, 3H, CH_2-CH_3); 2.75−2.12 (several m, C$H_2CH_2CO_2$H); 0.76−1.30 [several m, 10H, $(CH_2)_5$] 0.43 and −1.78 (each s, 1H, NH). Mass calcd for: $C_{39}H_{48}N_4O_4$: 636. Found: 637 (M + 1).

Pyropheophorbide-*a* (6). Chlorin e_6 trimethyl ester **5** (350 mg, 0.549 mmol) was dissolved in dry 2,4,6-collidine (30 mL) and then carefully degassed with nitrogen at 50 °C under vacuum. Potassium *tert*-butoxide (Aldrich, 5.0 mL, 1 M in *tert*-butyl alcohol, 5.0 mmol) was added. The initial bright green color immediately turned orange, and the

(12) The pK_a data are taken from Dean, J. A. *Lange's Handbook of Chemistry*, 14th ed.; McGraw-Hill: New York, 1992; pp 8.19−8.71.

(13) Pandey, R. K.; Sumlin, A. B.; Constantine, S.; Aoudia, M.; Potter, W. R.; Bellnier, D. A.; Henderson, B. W.; Rodgers, M. A.; Smith, K. M.; Dougherty, T. J. *Photochem. Photobiol*. **1996**, *64*, 194−204.

reaction mixture was left stirring at room temperature for 20 min and was then quenched with degassed glacial acetic acid (10 mL). The flask was then connected to a small distillation assembly (condenser, receiving head, and a flask), and the acetic acid along with a small amount of collidine (5 mL) was removed under high vacuum at about 175 °C. The distillation assembly was dismantled, and fresh collidine (15 mL) was added. The reaction flask was then connected to a condenser, and the reaction mixture was heated at reflux (ca. 175 °C) under nitrogen for 2 h. The solvent was again removed as above. The residue so obtained was redissolved in dichloromethane (100 mL), washed with water (2 × 100 mL), and dried over anhydrous sodium sulfate (5 g). Evaporation of the solvent (30 °C, 20 Torr) gave **6** (as carboxylic acid) in 85% yield after recrystallization in a manner similar to that for **4** above: ^{1}H NMR ($CDCl_3$, δ ppm): 9.35 and 9.15 and 8.50 (each s, 1H, meso H); 7.80 (m, 1H, C*H*═CH_2); 6.25, 6.10 (each d, 1H, CH−C*H*$_2$); 5.22 (dd, 2H, −CH_2, exocyclic ring); 4.41 (q, 1H, 18H); 4.28 (d, 1H, 17H); 3.75 (q, 2H, C*H*$_2$$CH_3$ merged with one of the ring CH_3); 3.62, 3.35 and 3.10 (each s, 3H, ring CH_3); 2.80−2.10 (several m, C*H*$_2$*CH*$_2$$CO_2H$); 1.80 (d, 3H, 18-$CH_3$): 1.60 (t, 3H, CH_2C*H*$_3$); −1.78 (each s, 1H, NH).

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