

The Synthesis of Chlorophyll-*a* Skeleton Homologs Bearing Linear Six-Carbon Chain from Methyl Pheophorbide-*a*

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(Received 21 December 2010, Accepted 24 February 2011)

The synthesis of chlorins bearing 6C-straight chain was accomplished from methyl pheophorbide-*a*. A series of chemical modifications were employed for the construction of active functional groups, such as bromine atom or aldehyde group, on the periphery of chlorins. These functionalized chlorins further reacted with the reagent containing straight carbon-chain by common chemical reactions to produce target compounds. The structures of all new chlorins were characterized by elemental analysis, IR, UV-Vis and ¹H NMR spectra.

Keywords: Chlorophyll-*a*, Chlorin, Synthesis, Linear six-carbon chain, Photodynamic therapy (PDT)

INTRODUCTION

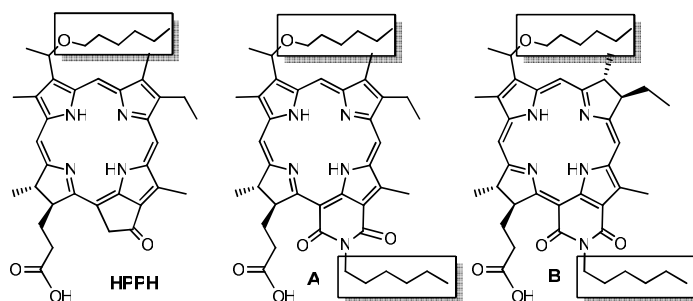
Photodynamic therapy (PDT) is a relatively new treatment method for the destruction of tumors, hyper-proliferative tissues, or other undesired structures. PDT is based on the accumulation of a photosensitizer in the malignant tissue after administration. Subsequent illumination with the light of an appropriate wavelength brings about a photochemical reaction to produce singlet oxygen (¹O₂) that results in the destruction of tumors or other tissues [1]. Different varieties of photosensitizers play a very important role in the process of PDT and their synthesis and modification are increasingly attracting scientists' attention [2]. Nowadays, there is a growing tendency for the development of new anticancer drugs associated with PDT to concentrate on the molecular design, chemical synthesis and biological studies on natural

chlorins due to their substantially stabilized *S*₁ energies, strong Q_y absorption bands, and unique redox reactivities [3].

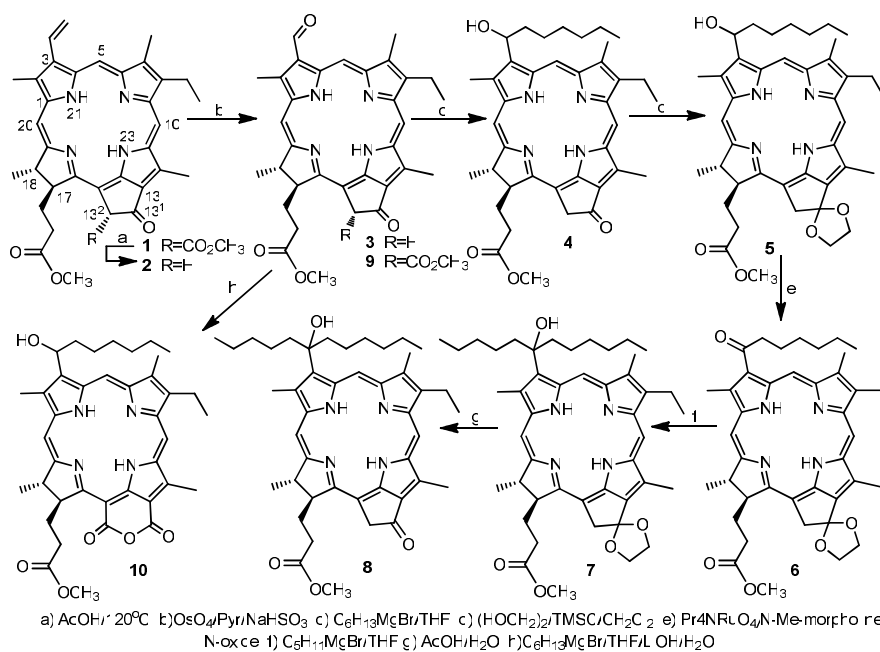
Many experimental data have indicated that the presence and position of substituents in the parent molecules make a remarkable difference in their biological activities. In a congeneric series of the alkyl ether derivatives of pyropheophorbide-*a*, it was found that the *in vivo* photodynamic efficacy extremely increased by introducing a linear six-carbon chain on the periphery of chlorin, regardless of alkyl or alkoxy moiety [4]. For example, 3-(1-hexoxylethyl)-pyropheophorbide-*a* (HPPH) has been utilized therapeutically, and some other photosensitizers are under clinical or preclinical trials, such as purpurinimide **A** and bacteriopurpurinimide **B** bearing two hexyl groups, which show excellent efficacy of PDT compared with the homologues of other alkyl group at the same position (Scheme 1) [5].

These research works suggest that the establishment and

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Scheme 1



Scheme 2

introduction of *n*-hexyl group at different positions of macrocyclic ring should provide some valuable information for developing new photosensitizers used in PDT. In this paper, a series of chlorins bearing linear 6C-chain were synthesized from methyl pheophorbide-*a*, as a degraded product of chlorophyll-*a*, by the chemical modifications along N^{21} - N^{23} axis (Schemes 2 and 3).

EXPERIMENTAL

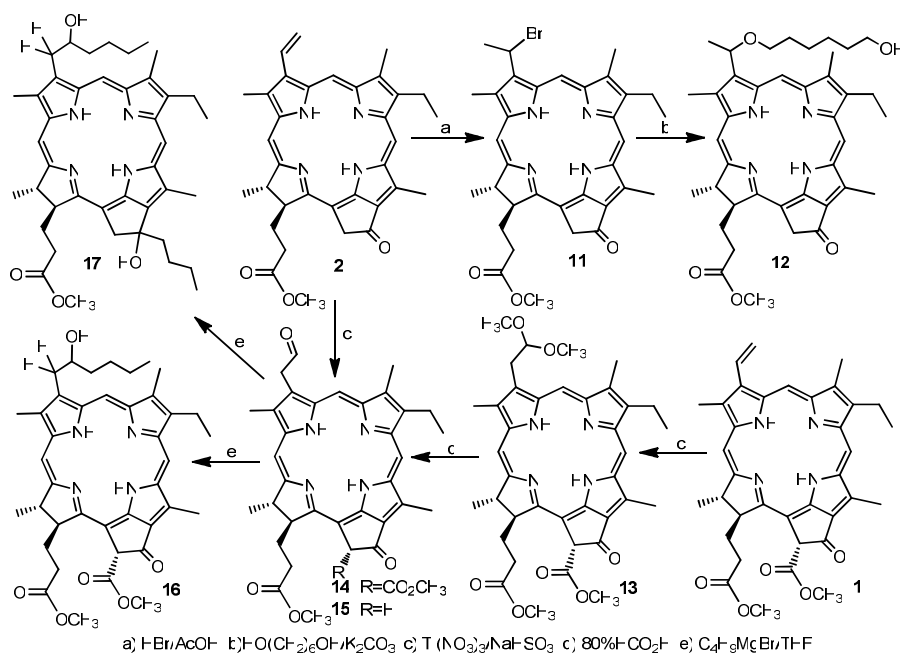
Melting points were determined with an Electrothermal 9100 apparatus and uncorrected. IR spectra were measured

with a Shimadzu FT IR 8300 spectrophotometer. The UV-Vis spectra were taken with a Unicam SP 800 spectrophotometer. The ^1H NMR spectra were recorded on a Bruker ARX-400 using TMS as internal standard. The elemental analyses were performed on a Perkin-Elmer 240 microanalyzer. All chemical reagents were commercially available and were purified by using standard methods. Solvents were dried in routine ways and redistilled. Methyl pheophorbide-*a* (MPa) **1** was obtained according to Smith's method [6].

Methyl Porphyrin (MPPa, 2)

Methyl pheophorbide-*a* **1** (300 mg, 0.495 mmol) was

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Scheme 3

dissolved in acetic acid (30 ml) and refluxed under nitrogen in dark for 3 h. The mixture was poured into water, extracted with CH_2Cl_2 (2×50 ml), evaporated to dryness under vacuum and purified on chromatography on a silica gel column with hexane-ethyl acetate (3:1) as eluent to give **2** (220 mg, 81%) as a dark green solid. The analytical data were consistent with the ones in the literature [6].

Methyl Pyropheophorbide-*d* (MPPd, **3**)

To a THF solution (15 ml) of **2** (186 mg, 0.339 mmol), 0.5 ml pyridine and osmium(VIII) oxide (85 mg, 0.334 mmol) in 2 ml THF were added at 0°C , respectively. After stirring for 30 min at the same temperature, the reactive system was heated to room temperature and stirred for an additional hour. To the resultant mixture, an excess of a solution of sodium hydrogensulfite (15 g) in a 50% MeOH was added and stirred for 20 min. After filtering out the brown osmium(IV) oxide precipitate, CH_2Cl_2 (20 ml) and water (20 ml) were added to the mixture. The organic layer was separated and dried over anhydrous Na_2SO_4 . The solvent was removed to give the solid material that was suspended in a mixture of THF (15 ml) and silica gel (2.5 g). After addition of a solution of sodium metaperiodate (1 g) in water (15 ml), the color of the solution

changed from green to bronze within 30 min. After adding CH_2Cl_2 (20 ml), the mixture was filtered through cotton wool and then the resultant crude material was chromatographed on silica gel with hexane-ethyl acetate (3:1) as eluent to give **3** (161 mg, 86%) as a dark red solid. The analytical data were consistent with the ones in the literature [7].

Methyl-3-formyl-3-devinylpyropheophorbide-*a* (**9**)

This compound **9** was obtained from MPa **1** by same method for preparing chlorin **3**. Its analytical data consistent with ones in the literature [8].

3-(1-Hydroxyheptyl)-3-devinylpyropheophorbide-*a* (**4**)

To a solution of compound **3** (154 mg, 0.280 mmol) in THF (15 ml) at 0°C was added 0.35 ml of hexyl magnesium bromide in THF (1 M). The mixture was then allowed to stir for 25 min until it was poured to ice-cooled NH_4Cl solution. The aqueous phase was extracted with ether (2×15 ml). The combined organic layers were dried over anhydrous Na_2SO_4 .

After evaporation of the solvent, the residue was chromatographed with a silica gel column with hexane-ethyl acetate (3:1) to give methyl **4** (112 mg, 63%) as a green solid. m.p.: $219\text{--}221^\circ\text{C}$. UV-Vis (CHCl_3) λ_{max} : 409 (relative intensity, 1.00), 504 (0.12), 535 (0.10), 604 (0.08), 661 (0.45)

nm; ^1H NMR (CDCl_3) δ : -2.02 (br, H, 1H, NH), 0.33 (br, 1H, NH), 0.81 (d, $J = 7.4$ Hz, 3H, 3_F-H), 1.33~1.66 (m, 6H, 3_c-e-H), 1.67 (t, $J = 7.7$ Hz, 3H, 8b-H), 1.78 (d, $J = 7.4$ Hz, 3H, 18-CH₃), 2.02~2.40 (m, 2H, 17a-H), 2.41~2.81 (m, 4H, 17b-H+3b-H), 3.19, 3.35, 3.59, 3.61 (each s, each 3H, CH₃+OCH₃), 3.64 (q, $J = 7.7$ Hz, 2H, 8a-H), 4.11~4.33 (m, 1H, 17-H), 4.35~4.60 (m, 1H, 18-H), 4.99 (d, $J = 19.0$ Hz, 1H, 13²-H), 5.13 (d, $J = 19.0$ Hz, 1H, 13²-H), 6.08~6.18 (m, 1H, 3a-H), 8.45, 9.39, 9.59 (each s, each 1H, *meso*-H); ^{13}C NMR (100 MHz, CDCl_3): 192.0, 191.9, 174.0, 173.5, 172.7, 172.4, 162.4 (2C), 161.8 (2C), 155.5, 155.4, 151.0, 150.9, 150.2, 150.0, 145.2 (2C), 142.1, 142.0, 137.8, 137.7, 136.5, 136.4 (2C), 136.3, 136.2 (2C), 131.9, 131.8, 129.6, 129.4, 129.0 (2C), 126.2, 122.9 (2C), 122.3, 107.6 (2C), 104.3, 104.2, 98.0, 97.8, 93.6, 93.4, 89.1, 88.9, 53.8, 53.4, 51.8 (2C), 51.3, 50.8, 50.3, 50.2, 31.4 (2C), 31.1, 31.0, 30.2 (2C), 22.7 (2C), 19.5, 19.4, 17.6, 17.4 (2C), 12.3 (2C), 12.1 (2C), 11.3 (2C), 11.2, 10.8 (2C). IR (KBr) ν : 3446 (O-H), 2925, 2856 (C-H), 1741, 1688 (C=O), 1612 (C=C), 1533 (chlorin skeleton), 1463, 1307, 1132, 1076, 1000 cm^{-1} . Anal. Calcd. for $\text{C}_{39}\text{H}_{48}\text{N}_4\text{O}_4$: C, 73.56; H, 7.60; N, 8.80; found: C, 73.44; H, 7.51; N, 8.71.

3-(1-Hydroxylheptyl)-13¹-ethylenedioxy-3-devinyl pyropheophorbide-a (5). To a solution of the alcohol chlorin **4** (105 mg, 0.165 mmol) in dried CH_2Cl_2 (100 ml), 10 ml ethylene glycol and 1.5 ml trimethylsilyl chloride were added at room temperature, respectively. The resulted mixture was stirred in dark for 12 h and poured into 5% ammonia water. The separated organic layers were washed with water, dried over anhydrous Na_2SO_4 and evaporated to dry. The residue was chromatographed with a silica gel column with hexane-ethyl acetate (4:1) to give methyl **5** (76 mg, 68%) as a green solid. m.p.: 218~221 °C. UV-Vis (CHCl_3) λ_{max} : 395 (relative intensity, 1.00), 493 (0.12), 533 (0.05), 592 (0.05), 647 (0.29) nm; ^1H NMR (CDCl_3) δ : -3.26 (br, 1H, NH), -1.40 (br 1H, NH), 0.77 (t, $J = 7.2$ Hz, 3f-CH₃), 1.30~1.75 (m, 6H, 3_c-e-H), 1.68 (t, $J = 7.5$ Hz, 3H, 8b-H), 1.76 (d, $J = 7.1$ Hz, 3H, 18-CH₃), 2.03~2.34 (m, 2H, 17a-H), 2.38~2.77 (m, 4H, 17b-H+3b-H), 3.32, 3.45, 3.57, 3.57 (each s, each 3H, CH₃+OCH₃), 3.77 (q, $J = 7.5$ Hz, 2H, 8a-H), 4.40~4.50 (m, $J = 7.2$ Hz, 1H, 17-H), 4.54~4.66 (m, 5H, 18-H+OCH₂CH₂O), 5.00 (d, $J = 16.0$ Hz, 1H, 13²-H), 5.12 (d, $J = 16.0$ Hz, 1H, 13²-H), 6.29 (t, $J = 7.1$ Hz, 1H, 3a-H), 8.76, 9.61, 9.95 (each s, each 1H, *meso*-

H); IR (KBr) ν : 3446 (O-H), 2960, 2927, 2858 (C-H), 1737 (C=O), 1674 (C=C), 1542 (chlorin skeleton), 1523, 1458, 1398, 1174, 1068, 1051 cm^{-1} ; Anal. Calcd. for $\text{C}_{41}\text{H}_{52}\text{N}_4\text{O}_5$: C, 72.32; H, 7.70; N, 8.23; found: C, 72.24; H, 7.87; N, 8.36.

3-Heptanoyl-13¹-ethylenedioxy-3-devinyl pyropheophorbide-a (6). A mixture of **5** (120 mg, 0.176 mmol) and *N*-methylmorpholine *N*-oxide (40 mg) in CH_2Cl_2 (25 ml) was stirred for 15 min at room temperature under nitrogen. To resulted solution, 15 mg of tetrapropyl-ammonium perruthenate (TPAP) was added and stirred for additional 1 h. The reactive system was washed with water, dried over anhydrous Na_2SO_4 and evaporated to dry. The residue was chromatographed on silica gel initially with CH_2Cl_2 to remove excess *N*-methylmorpholine *N*-oxide, and then with 1% MeOH/ CH_2Cl_2 to obtain **6** (78 mg, 65%) as a red solid. m.p.: 204~207 °C. UV-Vis (CHCl_3) λ_{max} : 404 (relative intensity, 1.00), 505 (0.16), 540 (0.11), 611 (0.08), 663 (0.27) nm; ^1H NMR (CDCl_3) δ : -3.08 (br, 1H, NH), -1.34 (br 1H, NH), 0.92 (t, $J = 7.0$ Hz, 3g-CH₃), 1.39~1.66 (m, 8H, 3_c-f-H), 1.70 (t, $J = 7.6$ Hz, 3H, 8b-H), 1.78 (d, $J = 7.3$ Hz, 3H, 18-CH₃), 2.09~2.84 (m, 6H, 17a+17b+3b-H), 3.34, 3.54, 3.57, 3.68 (each s, each 3H, CH₃+OCH₃), 3.80 (q, $J = 7.5$ Hz, 2H, 8a-H), 4.36~4.46 (m, 1H, 17-H), 4.48~4.63 (m, 5H, 18-H+OCH₂CH₂O), 5.00 (d, $J = 15.9$ Hz, 1H, 13²-H), 5.14 (d, $J = 15.9$ Hz, 1H, 13²-H), 8.91, 9.60, 10.13 (each s, each 1H, *meso*-H); ^{13}C NMR (100 MHz, CDCl_3): 190.0, 173.6, 173.0, 168.2, 162.4, 155.7, 151.3, 150.4, 150.1, 148.5, 145.6, 142.4, 137.9, 137.7, 136.9, 136.7, 136.5, 132.1, 129.5, 129.2, 128.2 (2C), 123.6 (2C), 123.3, 104.8, 98.5, 93.6, 53.2, 51.5, 51.0, 50.3, 30.7, 30.6, 23.1, 19.7, 17.7, 12.6, 12.3, 11.5, 10.8. IR (KBr) ν : 3409, 3303 (O-H or N-H), 2960, 2929, 2866 (C-H), 1726, 1703 (C=O), 1647 (C=C), 1550 (chlorin skeleton), 1460, 1380, 1271, 1205, 1143, 1070 cm^{-1} . Anal. Calcd. for $\text{C}_{41}\text{H}_{50}\text{N}_4\text{O}_5$: C, 72.54; H, 7.42; N, 8.25; found: C, 72.44; H, 7.57; N, 8.09.

3-(1-Hydroxyl-1-pentylhexyl)-13¹-ethylenedioxy-3-devinyl pyropheophorbide-a (7). This compound **7** (59%) as a dark green solid was obtained from **6** by same method for preparing chlorin **4**. m.p.: 165~168 °C; UV-Vis (CHCl_3) λ_{max} : 395 (relative intensity, 1.00), 496 (0.11), 524 (0.05), 592 (0.05), 646 (0.30) nm; ^1H NMR (CDCl_3) δ : -3.10 (br, 1H, NH), -1.14 (br, 1H, NH), 0.71 (t, $J = 7.0$ Hz, 3H, 3-CH₃), 0.73 (t, $J = 6.9$ Hz, 3H, 3-CH₃), 1.12~1.90 (m, 14H, 3-CH₂-), 1.74

(t, $J = 7.6$ Hz, 3H, 8b-H), 1.80 (d, $J = 7.2$ Hz, 3H, 18-CH₃), 2.19~2.79 (m, 8H, 17a+17b-H+HOCCH₂-), 3.40, 3.61, 3.65, 3.68 (each s, each 3H, CH₃+OCH₃), 3.80 (q, $J = 7.6$ Hz, 2H, 8a-H), 4.48~4.73 (m, 6H, 17-H+18-H+OCH₂CH₂O), 5.02 (d, $J = 15.8$ Hz, 1H, 13²-H), 5.17 (d, $J = 15.8$ Hz, 1H, 13²-H), 8.87, 9.71, 10.52 (each s, each 1H, *meso*-H); IR (KBr) ν : 3425, 3334 (O-H or N-H), 2920, 2850 (C-H), 1733 (C=O), 1616 (C=C), 1585 (chlorin skeleton), 1490, 1440, 1257, 1164, 1085, 1006 cm⁻¹. Anal. Calcd. for C₄₆H₆₂N₄O₅: C, 73.57; H, 8.32; N, 7.46; found: C, 73.68; H, 8.43; N, 7.30.

3-(1-Hydroxyl-1-pentylhexyl)-3-devinylpyropheophorbide-*a* (8). A solution of compound **7** (88 mg, 0.117 mmol) in 60% acetic acid (25 ml) was stirred at 45 °C in dark for 1 h. The mixture was poured into ice-cooled water and extracted with CH₂Cl₂ (2 × 15 ml). After washing with water, the organic layer was dried over anhydrous Na₂SO₄. The crude product was purified with a silica gel column with hexane-ethyl acetate (3:1), removing of the solvent, to give **8** (74 mg, 90%) as a dark green solid; m.p.: 235~238 °C. UV-Vis (CHCl₃) λ_{\max} : 409 (relative intensity, 1.00), 504 (0.11), 535 (0.10), 604 (0.07), 660 (0.43) nm; ¹H NMR (CDCl₃) δ : -1.78 (br, H, NH), 0.31 (br, 1H, NH), 0.77 (t, $J = 7.0$ Hz, 3H, 3-CH₃), 0.87 (t, $J = 6.9$ Hz, 3H, 3-CH₃), 1.10~1.65 (m, 14H, 3-CH₂-), 1.67 (t, $J = 7.5$ Hz, 3H, 8b-H), 1.78 (d, $J = 7.2$ Hz, 3H, 18-CH₃), 2.02~2.75 (m, 8H, 17a-H+17b-H+3-HOCCH₂-), 3.22, 3.46, 3.59, 3.64 (each s, each 3H, CH₃+OCH₃), 3.68 (q, $J = 7.5$ Hz, 2H, 8a-H), 4.21~4.28 (m, 1H, 17-H), 4.43 (q, $J = 7.4$ Hz, 1H, 18-H), 5.05 (d, $J = 19.9$ Hz, 1H, 13²-H), 5.22 (d, $J = 19.9$ Hz, 1H, 13²-H), 8.52, 9.47, 10.14 (each s, each 1H, *meso*-H); ¹³C NMR (100 MHz, CDCl₃): 190.0, 173.6, 173.0, 168.2, 162.4, 155.7, 151.3, 150.3, 150.1 (2C), 148.5, 145.6, 142.4, 137.9, 137.6, 136.9, 136.7, 136.5, 132.1, 129.2, 126.2, 128.1 (2C), 123.6 (2C), 123.2, 104.8, 98.5, 97.3, 93.6, 64.9, 53.2, 51.5, 51.4, 30.7, 30.5, 23.3, 23.1, 19.7, 17.7, 12.6, 12.3, 11.5, 10.8. IR (KBr) ν : 3465, 3323 (O-H or N-H), 2956, 2920, 2850 (C-H), 1735, 1697 (C=O) 1605 (C=C), 1502 (chlorin skeleton), 1458, 1444, 1263, 1189, 1083, 1043 cm⁻¹; Anal. Calcd. for C₄₄H₅₈N₄O₄: C, 74.75; H, 8.27; N, 7.93; found: C, 74.59; H, 8.35; N, 8.05.

3-(1-Hydroxylhexyl)-3-devinyl purpurin-18 methyl ester (10). To a solution of compound **9** (165 mg, 0.271 mmol) in THF (15 ml) at 0 °C was added 0.35 ml of hexyl magnesium bromide in THF (1 M). The mixture was then allowed to stir for 25 min until it was poured to ice-cooled

saturated MeOH with KOH, and the mixture was stirred in open system in dark for 2 h, poured into cool water, adjusted pH to 3 with hydrochloric acid and then extracted with CH₂Cl₂ (2 × 15 ml). The combined extracts was washed with water, dried over anhydrous Na₂SO₄ and concentrated to dryness. The resulted solution was treated with ethereal diazomethane and evaporated to remove the solvent. The residue was purified by using chromatography on a silica gel column with hexane-ethyl acetate (4:1) to give **10** as a red solid (74 mg, 41%). m.p.: 244~247 °C. UV-Vis (CHCl₃) λ_{\max} : 410 (relative intensity, 1.00), 477 (0.05), 505 (0.08), 542 (0.18), 657 (0.10), 693 (0.37) nm; ¹H NMR (CDCl₃) δ : -0.32 (br, H, NH), -0.20 (br, 1H, NH), 0.86 (t, $J = 6.9$ Hz, 3H, 3-CH₃), 1.10~1.70 (m, 14H, 3-CH₂-), 1.68 (t, $J = 7.5$ Hz, 3H, 8b-H), 1.72 (d, $J = 7.2$ Hz, 3H, 18-CH₃), 2.12~2.98 (m, 6H, 17a+17b-H+3-HOCCH₂-), 3.15, 3.32, 3.59, 3.61 (each s, each 3H, CH₃+OCH₃), 3.54 (q, $J = 7.5$ Hz, 2H, 8a-H), 4.21~4.37 (m, 1H, 18-H), 4.98~5.18 (m, 1H, 17-H), 6.06~6.14 (m, 1H, 3a-H), 8.47, 9.32, 9.68 (each s, each 1H, *meso*-H); IR (KBr) ν 3469, 3344 (O-H, or N-H), 2862, 2927 (C-H), 1749, 1747, 1704 (C=O), 1629 (C=C), 1591 (chlorin skeleton), 1491, 1448, 1263, 1087, 1041 cm⁻¹. Anal. Calcd. for C₃₉H₄₆N₄O₆: C, 70.25; H, 6.95; N, 8.40; found: C, 70.37; H, 6.82; N, 8.28.

3-[1-(6-Hydroxylhexyl)ethyl]-3-devinyl pyropheophorbide-*a* methyl ester (12). A solution of compound **2** (300 mg, 0.547 mmol) in 30% hydrogen bromide in acetic acid (20 ml) was stirred at room temperature under nitrogen in dark for 5 h. After the solvent was removed under reduced pressure the residue was redissolved in dried CH₂Cl₂ (20 ml), and anhydrous K₂CO₃ (80 mg) and 1,6-hexanediol (150 mg) were rapidly added to this solution under nitrogen. The resulted mixture was allowed to stir at ambient temperature in dark for 10 h and poured into ice-cooled water and extracted with CH₂Cl₂. After washing with water, the organic layer was dried over anhydrous Na₂SO₄. The crude product was purified with a silica gel column with hexane-ethyl acetate (1:1) to give **12** (186 mg, 51%) as a dark green solid; m.p.: 255~258 °C. UV-Vis (CHCl₃) λ_{\max} : 409 (relative intensity, 1.00), 473 (0.07), 505 (0.13), 535 (0.12), 605 (0.11), 661 (0.50) nm; ¹H NMR (CDCl₃) δ : -1.79 (br, 1H, NH), 1.15 (br, 1H, NH), 1.16~1.75 (m, 8H, 3-CH₂-), 1.64 (t, $J = 7.6$ Hz, 3H, 8b-H), 1.75 (d, $J = 7.2$ Hz, 3H, 18-CH₃), 2.06 (d, $J = 6.6$ Hz, 3H, 3a-CH₃), 2.10~2.70 (m, 6H, 17a+17b-H+3-HOC(CH₃)CH₂-), 3.21, 3.32, 3.54, 3.60 (each s, each 3H, CH₃+OCH₃), 3.42~3.78 (m, 4H,

HOCH₂+8a-H), 4.21 (d, *J* = 8.5 Hz, 1H, 17-H), 4.42 (q, *J* = 7.3 Hz, 1H, 18-H), 5.09 (d, *J* = 19.8 Hz, 1H, 13²-H), 5.17 (d, *J* = 19.8 Hz, 1H, 13²-H), 5.84 (q, *J* = 6.6 Hz, 1H, 3a-H), 8.46, 9.44, 9.71 (each s, each 1H, *meso*-H); IR (KBr) *v*: 3458 (O-H), 2958, 2856 (C-H), 1737, 1704 (C=O), 1616 (C=C, 1571 (chlorin skeleton), 1481, 1438, 1265, 1081, 1022 cm⁻¹. Anal. Calcd. for C₄₀H₅₀N₄O₅: C, 72.04; H, 7.56; N, 8.40; found: C, 72.18; H, 7.59; N, 8.25.

3-Formylmethyl-3-devinyl pheophorbide-*a* methyl ester (14). To MPa **1** (250 mg, 0.412 mmol) in a mixture of CH₂Cl₂ (60 ml) and MeOH (10 ml), thallium(III) nitrate trihydrate (670 mg, 1.50 mmol) in MeOH (20 ml) was added rapidly. After stirring for 3 h, this solution was treated with SO₂ by bubbled through and concd HCl (1.5 ml). The white thallium(I) salts were filtered off and the filtrate was washed with water, dried over anhydrous Na₂SO₄ and evaporated to dry under vacuum to give crude product **13**. This residue was dissolved in 88% formic acid (20 ml) and stirred for 4 h, and 30 ml of CH₂Cl₂ and 20 ml of water were added to the reactive system. The organic layer was evaporated under vacuum. The residue was purified with a silica gel column with hexane-ethyl acetate (3:1) to give **14** (205 mg, 80%) as a dark green solid; m.p.: 210~213 °C. UV-Vis (CHCl₃) λ_{max}: 393 (relative intensity, 1.00), 472 (0.05), 503 (0.07), 533 (0.11), 556 (0.04), 605 (0.10), 662 (0.51) nm; ¹H NMR (CDCl₃) δ: -1.75 (br, 2H, NH), 1.63 (t, *J* = 7.4 Hz, 3H, 8-CH₃), 1.83 (d, *J* = 6.8 Hz, 3H, 18-CH₃), 2.27~2.29, 2.59~2.63 (each m, 4H, 17a+17b-H), 3.12, 3.26, 3.57, 3.63, 3.89 (each s, each 3H, CH₃+OCH₃), 3.61 (q, *J* = 7.2 Hz, 2H, 8-H), 4.22~4.24 (m, 1H, 17-H), 4.47~4.49 (m, 1H, 18-H), 4.75 (s, 2H, 3a-H), 6.25 (s, 1H, 13²-H), 8.55, 8.98, 9.39 (each s, each 1H, *meso*-H), 10.08 (s, 1H, CHO); IR (KBr) *v*: 3404 (N-H), 2958-2860 (C-H), 1737-1697 (C=O), 1618 (C=C), 1563 (chlorin skeleton), 1400, 1245, 1063, 1010 cm⁻¹. Anal. Calcd. for C₃₆H₃₈N₄O₆: C, 69.44; H, 6.15; N, 9.00; found: C, 69.59; H, 6.28; N, 8.90.

3-Formylmethyl-3-devinyl pyropheophorbide-*a* methyl ester (15). This compound **15** (83%) as a dark green solid was obtained from **2** by same method for preparing chlorin **14**. Its analytical data consistent with ones in the literature [9].

3-(2-Hydroxyhexyl)-3-devinyl pheophorbide-*a* methyl ester (16). To a solution of compound **14** (150 mg, 0.241 mmol) in THF (15 ml) at 0 °C was added 0.35 ml of pentyl magnesium bromide in THF (1 M). The mixture was then allowed to stir for 15 min until it was poured to ice-cooled

NH₄Cl solution. The aqueous phase was extracted with ether. The combined organic layers were dried over Na₂SO₄. After evaporation of the solvent, the residue was chromatographed with a silica gel column with hexane-ethyl acetate (3:1) to give methyl **4** (87 mg, 53%) as a green solid. m.p.: 219~221 °C. UV-Vis (CHCl₃) λ_{max}: 408 (relative intensity, 1.00), 503 (0.13), 533 (0.13), 602 (0.12), 658 (0.46) nm; ¹H NMR (CDCl₃) δ: -1.80 (br, 1H, NH), 0.44 (br, 1H, NH), 0.88 (br s, 3H, 3_F-H), 1.33~1.84 (m, 6H, 3c~e-H), 1.60 (t, *J* = 7.6 Hz, 3H, 8b-H), 1.74 (d, *J* = 7.4 Hz, 3H, 18-CH₃), 1.98~2.59 (m, 4H, 17a+17b-H), 3.10, 3.19, 3.46, 3.59, 3.85 (each s, each 3H, CH₃+OCH₃), 3.64 (q, *J* = 7.7 Hz, 2H, 8a-H), 3.65~3.80 (m, 2H, 3a-H), 3.97~4.17 (m, 1H, 3b-H), 4.20~4.45 (m, 2H, 17+18-H), 6.06 (s, 1H, 13²-H), 8.41, 9.03, 9.18 (each s, each 1H, *meso*-H); IR (KBr) *v*: 3421 (N-H), 2958, 2858 (C-H), 1739~1704 (C=O), 1612 (C=C). 1553 (chlorin skeleton), 1401, 1248, 1051, 1002 cm⁻¹. Anal. Calcd. for C₄₀H₄₈N₄O₆: C, 70.56; H, 7.11; N, 8.23; found: C, 70.44; H, 7.29; N, 8.38.

3-(2-Hydroxyhexyl)-3-devinyl pyropheophorbide-*a* methyl ester (17). This compound **17** (50%) as a dark green solid was obtained from **15** by the reaction with hexyl magnesium bromide by same method for preparing chlorin **14**. m.p.: 264~267 °C. UV-Vis (CHCl₃) λ_{max}: 397 (relative intensity, 1.00), 450 (0.11), 501 (0.04), 645 (0.21), 650 (0.46) nm; ¹H NMR (CDCl₃) δ: -3.34 (br, H, NH), -1.50 (br, 1H, NH), 0.85 (t, *J* = 6.9 Hz, 3H, -(HO)C(CH₂)₃CH₃), 0.90 (t, *J* = 6.4 Hz, 3H, -(HO)C(CH₂)₃CH₃), 1.30~1.90 (m, 8H, 3d~e-H+13¹b-c-H), 1.75 (t, *J* = 7.6 Hz, 3H, 8b-H), 1.81 (d, *J* = 7.4 Hz, 3H, 18-CH₃), 2.10~2.80 (m, 10H, 17a+17b-H+-(HO)CCH₂-), 3.41, 3.48, 3.38, 3.62 (each s, each 3H, CH₃+OCH₃), 3.83(q, *J* = 7.7 Hz, 2H, 8a-H), 3.98-4.28 (m, 2H, 3a+3b-H), 4.37~4.75 (m, 2H, 17+18-H), 4.98 (d, *J* = 16.2 Hz, 1H, 13²-H), 5.18 (d, *J* = 16.2 Hz, 1H, 13²-H), 8.87, 9.65, 9.71 (each s, each 1H, *meso*-H); IR(KBr) *v*: 3469, 3350 (O-H or N-H), 2964, 2929, 2869 (C-H), 1741 (C=O), 1606 (C=C), 1533 (chlorin skeleton), 1458, 1400, 1311, 1170, 1137, 1081, 1020 cm⁻¹; Anal. Calcd. for C₄₂H₅₆N₄O₄: C, 74.08; H, 8.29; N, 8.03; found: C, 74.24; H, 8.19; N, 8.18.

RESULTS AND DISCUSSIONS

In this experiment, methyl pheophorbide-*a* (MPa) **1**, was extracted from the alga *Spirulina* [6], and degraded into methyl pyropheophorbide-*a* (MPPa) **2** by decarbo-

methoxylation in refluxing in AcOH. The subsequent oxidization was effected with OsO₄ in THF containing catalytic pyridine at 0 °C and was followed by glycol cleavage with NaIO₄ in aqueous THF to give the methyl pyropheophorbide-*d* (MPPd) **3**. The introduction of 6C-aliphatic chain to the C3-position of chlorin **3** was completed by Grignard reaction with hexylmagnesium bromide in THF at 0 °C to form alcohol chlorin **4** bearing a *n*-hexyl group at 3a-position in 63% yield. To avoid the effect of exocyclic carbonyl group on the following reactions, this group was protected with ethylene glycol using trimethylsilyl chloride as the catalyst to give acetal **5** in 68% yield, which was oxidized with tetrapropylammonium perruthenate (TPAP) and *N*-methylmorpholine to produce chlorin **6** in 65% yield. In order to construct a linear 6C-alkoxyl moiety, the second alkyl was introduced into this chlorin chromophore by the said Grignard reaction with pentylmagnesium bromide in THF at room temperature to generate tertiary alcohol **7** in 59% yield which contained simultaneously 6C-alkyl group at 3a-position and 6C-alkoxy-structures linked to the 3-position. After deprotection under acidic condition, the dialkyl-substituted ketochlorin **8** was obtained in 90% yield. The aldehyde **9**, prepared analogously to the MPPd **3** from MPa **1**, was converted into 3a-hexyl-substituted purpurin-18 methyl ester **10** by Grignard reaction with hexylmagnesium bromide and allomerization in saturated methanol with LiOH [10].

Concerned that the chlorins, linked with 6C-aliphatic chain at different positions, might provide us with valuable information in the determination of their PDT activities, we attempted to introduce multi-formed carbon chain to the peripheries of chlorins. The additional reaction of MPPa **1** with 30% hydrobromic acid in acetic acid was triggered to give brominated chlorin **11**, which was treated with 1,6-hexanediol in the presence of K₂CO₃ to form chlorin **12** as diastereomeric mixture in 51% yield. In this molecule, a 6C-straight chain, connected with an oxygen atom at each end, was located in 3a-position. To introduce 2-hydroxylhexyl fragment as the other form of 6C-chain the C3-vinyl group was converted into formylmethyl group by a moderate oxidation reaction. First, the treatment of starting material **1** with thallium(III) nitrate in THF gave bisdimethylacetal **13** in good yield. Subsequently, the resultant methylal was hydrolyzed in 80% formic acid to release C3-formylmethyl-substituted chlorin **14** in 90% yield. The MPPa **2** was also

converted into the relative aldehyde chlorin **15** by adopting the same oxidation procedure as for preparing **14**. The Grignard reaction of **14** with butylmagnesium bromide in THF at 0 °C smoothly produced chlorin **16** with 2-hydroxylhexyl group at 3-position in 53% yield, while the corresponding reaction of chlorin **15** with butylmagnesium bromide gave diol chlorin **17** in 50% yield which was introduced as two alkyl groups at 3b- and 13¹-positions, respectively.

The chromophores of chlorins relating to chlorophyll-*a* belonged to aza-annulene aromatic structures and their ring current flowing along π -system caused intense shield and deshield effect to the functional group linked with inner ring and outer ring, respectively. The ¹H NMR spectrum of all chlorins clearly showed signals of protons, attached to the center nitrogen atom, in very high-field shift. On the contrary, the methyl groups bonded to chlorin periphery were strongly deshielded and moved to lower-field, whose chemical shifts were even very close to the methoxyl group away from chlorin chromophore at 17-position. The ¹³C NMR spectrum of chlorin **4** demonstrated complex signals which were ascribable to a pair of epimers, and those of chlorin **8** showed the corresponding number of carbons.

In summary, we have described a purposeful synthesis of chlorins bearing 6C-aliphatic straight chain by modifying their active reaction regions on the peripheries, such as the C3-vinyl group and the oxygen-containing functional groups of *E*-ring. Such reactions conveniently constructed 6C-straight chain moieties on the macrocycles resembling HPPH. These modifications of the parent ring of chlorophyll-*a* derivatives may be valuable in the generation of novel photosensitisers for PDT.

ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of Shandong Province of China (No. Y2008B49) and by the open project of state key laboratory breeding base of green chemistry-synthesis technology, Zhejiang University of Technology.

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