The Synthesis of Chlorin-based Photosensitizers for Using in Photodynamic Therapy by Modification of Methyl Pyropheophorbide-a

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The Grignard reaction of methyl pyropheophorbide-a (MPP-a) was performed to introduce lengthy alkyl chain for improving lipophilicity. After the introduction of alkyl chain to the both of carbonyls, peripheral aldehyde and cyclopentanone, the obtained diol **3** was subjected to dehydration to give monodehydrated product **4** selectively. The Qy band of prepared compounds were affected by the substituents on the Qy axis (N_{21}-N_{23}).

Key words: Chlorin-based porphyrin, methyl pyropheophorbide-a, Grignard reaction, Photodynamic therapy (PDT), photosensitizer

INTRODUCTION

Photodynamic therapy (PDT) is a medical treatment which employs the combination of light and a drug to bring about a cytotoxic or modifying effect to cancerous or other unwanted tissue. This procedure involves local photochemical activation following accumulation of the photosensitzer (drug) in tumor [1]. Photofrin II®, which has been used in worldwide for tumor photosensitization, meets the above criteria reasonably well but lacks rapid clearance from normal tissue including skin, thus rendering patients photosensitive for a month or longer after treatment. It has also the disadvantage of being chemically complex and its longest wavelength absorbance peak at 630 nm is therapeutically sub-optimal for tissue penetration [2].

In order to overcome these disadvantages, considerable effort has been put into the development of new photosensitizer, which should be a single compound, and should have improved tumor selectivity and increased absorption in red region for deeper tissue penetration of light and minimal skin photosensitivity to sunlight. During the last few years, a number of natural chlorin derivatives, which absorb strongly in the range above 650 nm, have been reported as potential photosensitizers [3]. Besides strong Qy absorption band, the substantially stabilized S_1 energies and unique redox reaction make natural chlorin pigments standing in marked contrast to symmetric porphyrin pigments. Many chlorin-based porphyrins have shown that overall lipophilicity of molecule plays a major role in tumor localization [4]. The lipophilic characteristics of chlorin molecules can be altered by varying the length of the carbon chain of the substituted alkyl group. These results

prompted us to prepare a series of alkylated chlorin derivatives for improving PDT efficacy.

For the preparation of the desired photosensitizers, methyl pyropheophorbide-a (MPP-a) was selected as a starting material for the following reasons: (a) the distortion caused by the presence of a fused cyclopentanone ring would help in varying symmetry of pyropheophorbide pigments in modifying exocyclic ketone group, (b) the making use of inherent reactive groups, such as vinyl group at 2-position and exocyclic ketone group at 9-position should change the overall lipophilicity by introducing the lengthy alkyl chain, (c) the reaction at 2- and 9-position, which largely influence on the Qy band, should exhibit some change in the visible spectra.

To introduce lengthy alkyl chain, an active functional groups was prepared initially. The treatment of MPP-a with Thallium(III) nitrate gives the bisdimethylacetal **1** which can either be isolated or stirred *in situ* at room temperature in 88% formic acid to generate aldehyde **2**. The Grignard reaction of **1** at 2- and 9-position with a straight chain Grignard reagent affords diol **3** which were subjected to dehydration with p-TsOH at 100°C in toluene to give an olefin **4**.

MATERIALS AND METHODS

Instruments. Ultra-violet (UV) spectra were taken on a Varian model Cary-1 Spectrophotometer. ^{1}H-nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 300 MHz NMR spectrometer.

Methyl pyropheophorbide-a was obtained from alga *Spirulina Maxima* according to K. M. Smith's method [5].

3-(2,2-Dimethoxyl)-3-devinylpyropheophorbide-a methyl ester (1) MPP-a (45 mg) was brought to a solution of CH_2Cl_2 (10 ml) and MeOH (2 ml). Thallium (III) nitrate trihydrate was dissolved in MeOH (5 ml) and was added rapidly at 0°C into the

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reaction vessel. The solution was maintained for 10 min and then saturated NaHSO₃ was added. After adding conc. HCl, the white thallium(I) salts were filtered off and the solution was diluted with CH₂Cl₂ and washed with water. The solvent was removed and the residue was chromatographed on silica gel column to give the title compound in 78% yield. NMR: δ 9.49, 9.27, 8.50 (each1H, s, meso-H), 5.25(1 H, d, J = 18.7 Hz, 10-H), 5.01(1 H, d, J = 18.7 Hz, 10-H), 5.01(1 H, t, 2b-H, 4.40, 4.20(each 2 H, m, 7,8-H), 4.10(2 H, d, 2a-H), 3.88, 3.60, 3.46,3.47, 3.30, 3.20(each 3 H, s, Me and OMe), 3.64(2 H, m, 4a-H), 2.60, 2.29(each 2 H, m, 7a, 7b-H), 1.78(3 H, d, J = 6.8 Hz, 8-H), 1.69(3 H, t, J = 7.6 Hz, 4b-H), -1.60(2 H, br, s, NH). Vis(CH₂Cl₂) λ_max = 657.6 nm (relative intensity, 2.67), 602.0(0.55), 534.2(0.60), 503.4(0.63), 471.4(0.28), 389.6(4.00).

3-(2-Methyformyl)-3-devinylpyropheophorbide-a methyl ester (2) The obtained compound **1** was dissolved in 88% formic acid (20 m*l*) and stirred for 6 hr. After removing the solvent, the residue was redissolved in CH₂Cl₂, washed with water, and the crude product was purified by using chromatography on silica gel column to give the title compound in 92% yield. NMR: δ 10.8(1 H, s, formyl-H), 9.35, 8.98, 8.55(each 1 H, s, meso-H), 5.25(1 H, d, J = 18.2 Hz, 10-H), 4.92(1 H, s, d, J = 18.2 Hz, 10-H), 4.75(2 H, s, 2a-H), 4.48, 4.23(each 1 H, m, 7,8-H), 3.89, 3.57, 3.56, 3.12(each 3 H, s, Me and O Me), 3.61(2 H, q, 4a-H), 2.62, 2.19(each 2 H, m, 7a, 7b-H), 1.83(3 H, d, J = 7.2 Hz, 8-H), 1.63 (3 H, t, J = 7.4 Hz, 4b-H), -2.01(2 H, br, NH). Vis(CH₂Cl₂) λ_max = 661.8 nm (relative intensity, 0.83), 605.4(0.16), 555.8(0.07), 533.4(0.17), 503.4(0.11), 472.4(0.08), 392.9(1.62).

3-Devinyl-3-(2-hydroxyheptyl)-13-hydroxyl-13-hexylpyropheophorbide-a methyl ester (3) Compound **2** (57 mg, 0.1 mmol) was dissolved in 15 mL THF and a solution (0.5 mL) of hexyl magnesium in THF(1.0M) was added with stirring at 0°C. The solution was stirred for 10 min and this reaction was quenched with ethyl acetate. The mixture was poured into saturated solution of NH₄Cl, the aqueous phase was extracted with several portion of CH₂Cl₂ and the combined organic phases were washed with water, dried over Na₂SO₄ and evaporated *in vacuuo* to dryness. The residue was purified by chromatography to give the title compound in 64% yield. NMR; δ 9.70, 9.64, 8.85(each 1 H, s, meso-H), 5.14(1 H, d, J = 18.9 Hz, 10-H), 4.97(1 H, dd, J = 18.9 Hz, 10-H), 4.62(1 H, m, 7-H), 4.45(1 H, m, 8-H), 4.20(2 H, dm, J = 12.4 Hz, 3a-H), 3.84(2 H, q, J = 7.6 Hz, 4a-H), 3.62, 3.60, 3.48, 3.38 (each 3 H, s, Me and O Me), 1.86(3 H, d, J = 7.4 Hz, 8-Me), 1.73 (3 H, t, J = 7.8 Hz, 4b-H), 1.15-1.98(20 H, m, alkyl-H), 0.91, 0.85(each 3 H, t, J = 6.9 Hz, -CH₂CH₃), -1.57, -3.38(each 1 H, br, s, NH). Vis(CH₂Cl₂) λ max = 644.0 nm (relative intensity, 0.52), 590.8(0.14), 542.4(0.12), 498.0(0.23), 395.2(1.92).

3-Devinyl-3-(2-hydroxyheptyl)-13-(1-hexenyl)-pyropheophorbide-a methyl ester (4) Compound 3 (30 mg) was dissolved in 15 m*l* dry toluene containing 10 mg of p-TsOH. The mixture was stirred for 3 h at room temperature. After 15 m*l* of saturated NaHCO₃ was added, the reaction solvent was extracted with several portion of ethyl acetate. The combined organic phase were washed with water, dried over Na₂SO₄ and evaporated *in vacuo* to dryness.

The residue was purified by chromatography to give the title compound in 46% yield. NMR; δ 9.72, 9.64, 8.86(each 1 H, s, meso-H), 6,77(1 H, m, 9b-H), 5.40(1 H, d, J = 20.1 Hz, 10-H), 5.25(1 H, d, J = 20.1 Hz, 10-H), 4.62, 4.42(each 1 H, m, 7,8-H), 4.16(1 H, m, 2b-H), 4.19(2 H, J = 12.4 hz, 2a-H), 3.87(2 H, q,
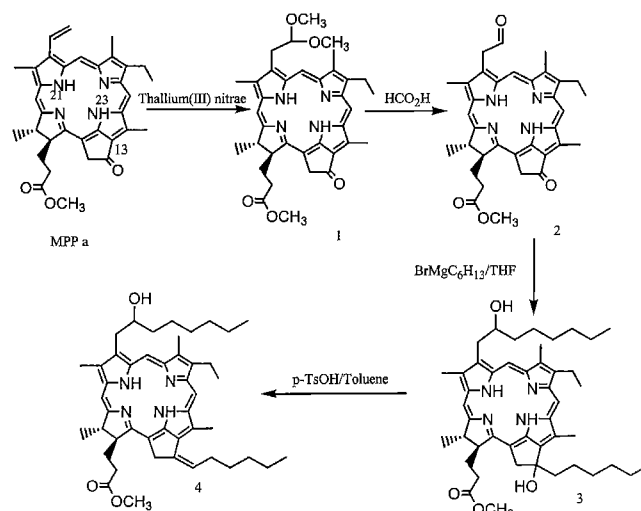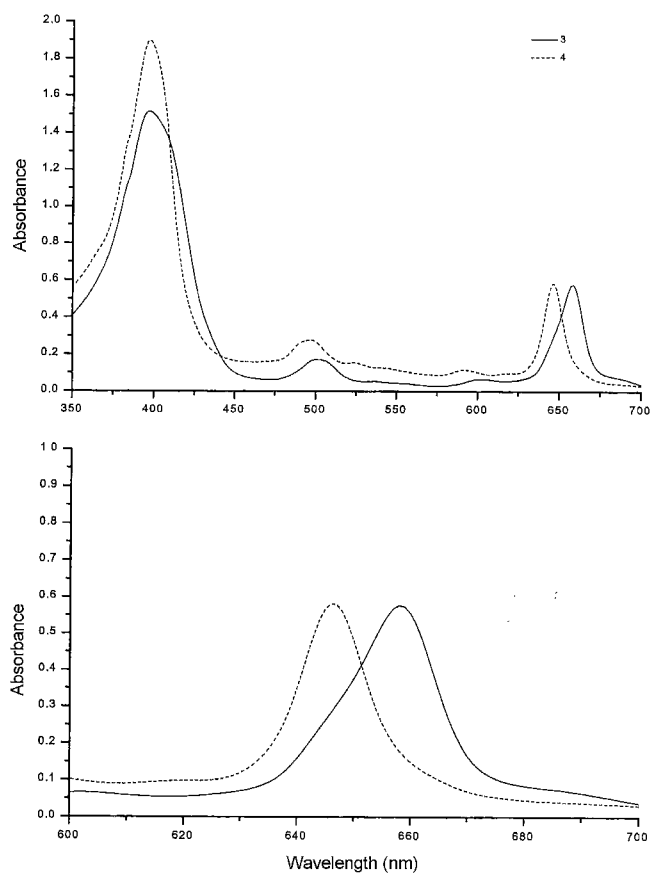

Scheme 1.

Figure 1. The Visible spectra of compound **3** and **4** in dichloromethane, normalized at Qy peak.

J = 7.6 Hz, 4b-H), 3.63, 3.56, 3.48, 3.41(each 3 H, s, Me and OMe), 2.72, 2.29(each 2 H, m, 8a,8b-H), 1.87(3 H, d, J = 7.4 Hz, 8-Me), 1.73(3 H, t, J = 7.8 Hz, 4b-H), 1.15-1.98(18 H, m, alkyl-H), 0,91, 0.85(each 3 H, t, J = 6.9 Hz, -CH$_2$CH$_3$), -1.57, -3.38 (each 1 H, br, s, NH). Vis(CH$_2$Cl$_2$) λmax = 666.0 nm (relative intensity, 0.78), 609.6(0.19), 538.6(0.219), 507.6(0.24), 412.6(2.11).

RESULTS AND DISCUSSION

The Grignard reaction into the carbonyl group is an important method for introducing long chain alkyl group. Like other aromatic ring, the carbonyl group conjugated with chlorin chromophore on the fused cyclopentane ring can take place this nucleophilic addition. For introducing longchain alkyl group to the peripheral group of macrocycle, acetaldehyde function was constructed by modifying vinyl group at 3-position to give aldehyde 2. Two alkyl chains were introduced by Grignard reaction. The obtained diol 3 was subjected to dehydration in toluene with p-TsOH. In this treatment, the dehydration was just happened to E-ring only to give the monodehydrated product 4, not to 2-position because the easiness of dehydration increases with a branching.

The visible spectra of porphyrin have been regarded as an important property. All porphyrin-like compounds have a strong absorption band around 400 nm called Soret band. Unfortunately, this band is not useful for PDT since blue light dose not penetrate very deeply into tissue; thus the weaker satellite absorption band (Qy-band) above 600 nm are useful for PDT treatment. In our research, the structure of chlorin at 2- and 13-position were modified and Qy band of compound were strongly affected by these subsitituents on Qy axis (N$_{21}$-N$_{23}$, see Scheme 1), i.e, functional group at 3- and 13-position. Bisdimethylacetal 1 and aldehyde 2 have the same devinyl structure at 3- position, thus Qy peak at 657 nm of 1 and 661 nm of 2 were blue-shifted in comparison with 668 nm of

MPP-a. This difference in visible spectra was explained by the fact that the vinyl group at 3-position were transformed to decrease conjugation region of peripheral substituted group with macrocycle. The comparison of Qy peak in compound 1 and 2 indicated that electron-withdrawing group without linking directly with chlorin chromophore hardly effected visible spectra of molecule. Diol 3 shown more weaker Qy absorption (644 nm) can be explained that two conjugative structures, vinyl and exocyclic ketone group, were converted into a single bond. After dehydration the Qy peak at 666 nm of 4 was red-shifted in comparison with with 644 nm of 3 because of forming olefin structure at 13^1-position to increase conjugation region (see Fig. 1).

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