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Synthesis of cationic water-soluble esters of chlorin e_6

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Abstract—Two esters of chlorin e_6 have been synthesized by esterification with aminoalcohols via the formation of acid chloride using oxalyl chloride and converted to the corresponding cationic water-soluble esters of chlorin e_6. The visible absorption and circular dichroism spectra have revealed that these two cationic chlorin e_6 esters synthesized are readily soluble in water as a monomer.

Chlorophylls and their derivatives have received much attention because of their potential usefulness as a second-generation photosensitizer of photodynamic therapy (PDT)[1] and an antimutagenic or an anticarcinogenic agent.[2] In view of their characteristic strong absorption in the so-called Soret and Q band regions and their selective accumulation on the cancer cells, the chlorophyll derivatives are very attractive as a photosensitizer in the next generation in place of some porphyrin derivatives currently used in PDT.[1] Among the chlorophyll derivatives, chlorin e_6, pheophorbide a and pyropheophorbide a have been most frequently used as an anionic photosensitizer for PDT. In recent years, cationic photosensitizers, for example, cationic porphyrins and phthalocyanines, have been reported to be highly useful for PDT,[3] although most chlorophyll derivatives so far synthesized with the aim of improving their tumor selectivity and therapeutic effectiveness are anionic.[4] Indeed, a few cationic chlorophyll derivatives have been synthesized,[5] but most of them are insoluble in water as a monomer. This is because all the cationic chlorophyll derivatives synthesized are mono-cationic and only slightly soluble in water and sometimes aggregated due to intermolecular π–π interaction. The self-aggregation of dye molecules might cause a deactivation of photoexcitation.[6] Therefore, we have designed two cationic water-soluble chlorin e_6 esters (**3** and **4**) in which three cationic ester substituents are introduced to the parent dye chlorin e_6, and they are expected to be soluble in water as a monomer.

In the previous reports, one, two, or three carboxyl groups of chlorin e_6 have been dealt with coupling reagents such as dicyclohexylcarbodiimide (DCC) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC) to form amide or ester bond.[7] However, the products are usually a mixture of mono-, di-, and tri-substituted chlorin e_6, mostly mono- and di-substituted chlorin e_6, probably because the structure of intermediates is too bulky to esterify all of the three carboxyl groups. This makes it difficult to isolate the desired product from the mixture of the esterified products. It is important to develop a synthetic method to introduce various functional substituents to the chlorin ring. Therefore, we have used oxalyl chloride[8] for esterification of chlorin e_6 in the present work. In this method, the intermediate, that is, acid chloride, is not bulky but reactive enough to build ester bonds sterically hindered, and the main product expected is tri-substituted chlorin e_6. In addition, the desired product may be easily separated because only volatile by-products are formed.

Chlorin e_6 was obtained by saponification of methyl pheophorbide a[9] according to the method of Hynninen.[10] Chlorin e_6 was esterified with the corresponding alcohol by using oxalyl chloride. Typically, a suspension of chlorin e_6 in anhydrous dichloromethane was refluxed under a dry nitrogen atmosphere. To the suspension was added an excess of fresh oxalyl chloride. The deep green solution obtained was refluxed with stirring for 15 min, followed by vaporization of dichloromethane and excess oxalyl chloride to give chlorin e_6 trichloride as a green paste. The green residue was re-dissolved in dry dichloromethane and refluxed under a nitrogen atmosphere. To the green solution obtained was carefully added the alcohol to prevent temperature rise of the reaction mixture and stirred at 45 °C for 1 h. The desired compound

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Keywords: Chlorophyll; Chlorin e_6; Water-soluble chlorin; Cationic chlorin; Oxalyl chloride; Esterification.

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was separated from the residue by column chromatography on alumina and evaporated to dryness to give the triester of chlorin e_6 as dark violet crystalline powder. In this research, new esters of chlorin e_6, that is, 6a-,γb-,7c-tris(2-dimethylaminoethyl) chlorin e_6 (**1**)[11] and 6a-,γb-,7c-tris(3-pyridylmethyl) chlorin e_6 (**2**),[12] were synthesized in yield of 34.8% and 13%, respectively (Fig. 1). In this reaction, the by-products were recognized, but, interestingly, they were neither di- nor mono-substituted chlorin e_6. In other words, the by-products have a methyl group at the γ position and thus are 6a-,7c-bis(2-dimethylaminoethyl) chlorin e_4 and 6a-,7c-bis(3-pyridylmethyl) chlorin e_4. This side reaction is known to be caused by boiling chlorin e_6 especially in basic conditions.[13] On the other hand, the yield of **1** is higher than that of **2**. The difference in yield between **1** and **2** is due to the fact that the pyridyl groups of **2** are bulky and rigid. As a result, oxalyl chloride is a good reagent for esterification of chlorin e_6 because the acid chloride is not bulky as an intermediate but is highly reactive. New cationic water-soluble chlorin e_6, for example, 6a-,γb-,7c-tris(2-trimethylammonioethyl) chlorin e_6 (**3**)[14] and 6a-,γb-,7c- tris(3-methylpyridinium-methyl) chlorin e_6 (**4**)[15] were synthesized by N-methylation of **1** and **2** in the usual manner. The structure of **1**–**4** was determined by ^{1}H NMR, ^{1}H–^{1}H COSY, FABMS, and elemental analysis.[11,12,14,15]

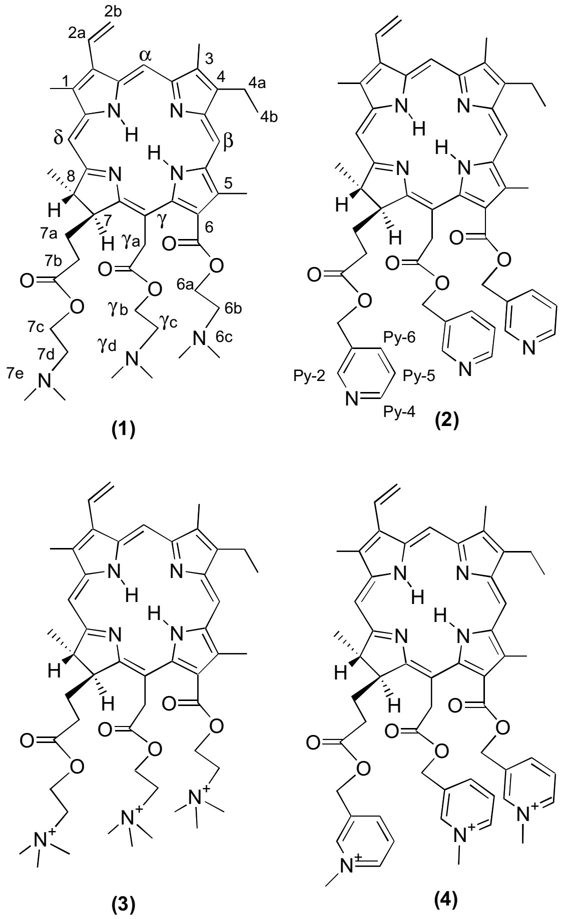

Figure 1. Structure of ester derivatives of chlorin e_6.

Compounds **3** and **4** were dissolved in Tris–HCl buffer at pH 7.6 and 25 °C. The absorption (vis) and circular dichroism (CD) spectra of **3** and **4** are shown in Figure 2. The vis spectra of **3** and **4** in the buffer have sharp Soret and Q bands and their CD spectra show a strong positive signal in the Soret region. These spectral features suggest that both **3** and **4** are soluble in the buffer as a monomer.[5,16,17] In fact, the spectra are hardly affected by temperature and ionic strength of the buffer. The temperature change of the buffer did not influence vis spectral parameters such as molar extinction coefficient ε and maximum absorption wavelength λ_{max} at the Soret or Q band in the range of 15–55 °C at all. As for the ionic strength, weak hypochromisity was observed for the Soret band of **3** and **4** upon increasing addition of NaCl. This hypochromic effect was very weak, for example, 6.0% for **3** and 4.7% for **4** in 60 mM NaCl and 7.4% for **3** and 6.2% for **4** in 100 mM NaCl. In addition, CD spectral patterns of **3** and **4** were hardly changed upon increasing addition of NaCl. The spectral features of **3** were quite similar to those of **4**. Thus, three cationic substituents hinder the intermolecular π–π interaction due to the electrostatic and/or steric repulsion which prevents **3** and **4** from self-stacking to make them soluble in the buffer as a monomer.

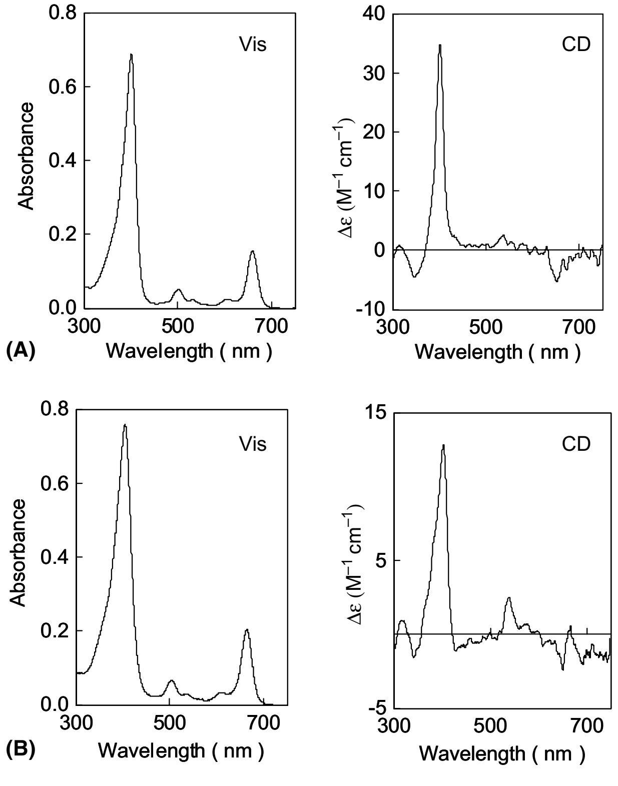

Figure 2. Vis and CD spectra of **3** (Panel A) and **4** (Panel B) in Tris–HCl buffer at pH 7.6 and 25 ° C. The concentration of **3** and **4** is 5.1 × 10^{-6} M and 8.0 × 10^{-6} M, respectively.

In conclusion, new water-soluble cationic esters of chlorin e_6 have been successfully synthesized for the first time. Oxalyl chloride is a good reagent for esterification of chlorin e_6. Tri-cationic **3** and **4** are soluble in water as a monomer since the three cationic substituents prevent **3** and **4** from self-stacking due to the electrostatic and/or steric repulsion.

Acknowledgements

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11. A suspension of 131 mg (0.213 mmol) chlorin e_6 in 30 mL anhydrous dichloromethane was refluxed under a nitrogen atmosphere. To the suspension was added 4.5 mL of fresh oxalyl chloride. The deep green solution obtained was refluxed with stirring for 15 min, followed by vaporization of dichloromethane and excess oxalyl chloride. The dark green residue was dissolved in 25 mL of dry dichloromethane and refluxed under a nitrogen atmosphere. To the green solution obtained was carefully added a few drops of 2-dimethylaminoethanol to prevent temperature rise of the reaction mixture. The rest of the total amount (7 mL) of 2-dimethylaminoethanol was added to the reaction mixture, followed by stirring at 45 °C for 1 h. The reaction mixture was evaporated to dryness and the residue obtained was dissolved in chloroform. The chloroform extract was washed with distilled water, dried with anhydrous sodium sulfate, and rotary-evaporated to dryness. The residue was chromatographed on basic alumina (Merck: Brockmann Grade V) using ethylacetate/*n*-hexane (1/1, v/v) containing 2% triethylamine as eluent. The main green band was collected and evaporated to dryness. The crystalline powder obtained was dissolved in a small amount of acetone and precipitated by pouring distilled water into the acetone solution. The precipitate collected by centrifugation was dried over diphosphorus pentaoxide in a vacuum desiccator to give **1** as dark violet crystals. Yield: 70.8 mg (8.64×10^{-2} mmol); 34.8%. ^{1}H NMR (300 MHz, $CDCl_3$): 9.68 (s, 1H, β-H), 9.55 (s, 1H, α-H), 8.73 (s, 1H, δ-H), 8.06 (dd, 1H, $J = 12$ Hz, 18 Hz, 2a-H), 6.34 (dd, 1H, $J = 1.2$ Hz, 17 Hz, 2b-H), 6.14 (dd, $J = 1.4$ Hz, 10 Hz, 2b′-H), 5.31 (m, 2H, γa-CH_2), 4.82 (m, 2H, 6b-CH_2), 4.43 (m, 2H, 7-H,8-H), 4.29 (t, 2H, $J = 6.1$ Hz, γc-CH_2), 4.06 (m, 2H, 7d-CH_2), 3.78 (q, 2H, $J = 7.6$ Hz, 4a-CH_2), 3.59 (s, 3H, 5a-CH_3), 3.47 (s, 3H, 1a-CH_3), 3.29 (s, 3H, 3a-CH_3), 2.94 (t, 2H, $J = 5.9$ Hz, 6a-CH_2), 2.58–2.37 (m, 2H, 7b-CH_2), 2.54 (m, 2H, γb-CH_2), 2.45 (s, 6H, 6c-CH_3, 6c′-CH_3), 2.39 (t, 2H, $J = 5.6$ Hz, 7c-CH_2), 2.25–2.05 (m, 2H, 7a-CH_2), 2.17 (s, 6H, γd-CH_3, γd′-CH_3), 2.10 (s, 6H, 7e-CH_3, 7e′-CH_3), 1.74 (d, 3H, $J = 7.1$ Hz, 8a-CH_3), 1.71 (t, 3H, $J = 7.6$ Hz, 4b-CH_3), −1.33 (br s, 1H, NH), −1.51 (br s, 1H, NH). FABMS (glycerol): *m*/*z*; 810 $[M+H]^+$. Anal. Calcd for $C_{46}H_{63}N_7O_6 \cdot 1/2H_2O$: C, 67.46; H, 7.88; N, 11.97. Found: C, 67.21; H, 7.78; N, 11.64.
12. A suspension of 29.4 mg (4.79×10^{-2} mmol) chlorin e_6 in 20 mL of anhydrous dichloromethane was refluxed under a nitrogen atmosphere. To the suspension was added 4.0 mL of oxalyl chloride and the reaction mixture was refluxed for 15 min. An excess of the oxalyl chloride and dichloromethane were vaporized. The dark green residue was dissolved in 10 mL of dry dichloromethane and refluxed under a nitrogen atmosphere. To the green solution was added a few drops of 3-pyridinemethanol. In about 15 min, about 4 mL of 3-pyridinemethanol and 1 mL of triethylamine were added to the solution and further stirred at 45 °C for 1 h. After the reaction mixture was rotary-evaporated to dryness, the residue was dissolved in chloroform and washed with distilled water. The chloroform layer was washed five times with water, dried with anhydrous sodium sulfate, and rotary-evaporated to dryness. The residue was chromatographed on basic alumina (Merck: Brockmann Grade V) using ethylacetate/methanol (100/2, v/v) containing 2% trimethylamine. A green main band was collected and evaporated to dryness. The crystalline powder obtained was dissolved in a small amount of acetone and precipitated from the acetone solution by addition of distilled water. The precipitate collected by centrifugation was dried over diphosphorus pentaoxide in a vacuum desiccator to give **2**

as dark violet crystals. Yield: 5.52 mg; 13%. ^{1}H NMR (300 MHz, CD_3OD): 9.34 (s, 1H, β-H); 9.10 (s, 1H, α-H); 8.76 (s, 1H, δ-H); 8.83, 8.57, 8.42, 8.38, 8.31, 8.09 (each, d or dd, 6H, py-2-H, py-6-H); 7.77 (dd, 1H, $J = 12$ Hz, 18 Hz, 2a-H); 7.52 (m, 3H, py-4-H); 7.19 (m, 3H, py-5-H); 6.11 (dd, 1H, $J = 0.72$ Hz, 18 Hz, 2b-H); 5.95 (dd, 1H, $J = 1.2$ Hz, 10 Hz, 2b′-H); 5.66 (m, 2H, γa-CH_2); 5.27 (s, 2H, 6a-CH_2); 5.14 (m, 2H, γb-CH_2); 4.37 (m, 2H, 7-H, 8-H); 3.33 (q, 2H, $J = 8.0$ Hz, 4a-CH_2); 3.33 (s, 3H, 5a-CH_3); 3.33 (s, 3H, 1a-CH_3); 3.28 (s, 3H, 3a-CH_3); 2.88 (s, 2H, 7c-CH_2); 2.53–2.95 (m, 2H, 7b-CH_2); 1.97–2.25 (m, 2H, 7a-CH_2); 1.55 (d, 3H, $J = 7.3$ Hz, 8a-CH_3); 1.50 (t, 3H, $J = 7.5$ Hz, 4b-CH_3). FABMS (glycerol); *m*/*z*: 870 (M+1).

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14. To 56.0 mg (6.91×10^{-2} mmol) **1** in 20 mL anhydrous acetone was added 10 mL of iodomethane after replacement of the atmosphere with nitrogen. The reaction mixture was stirred for 20 h at room temperature to afford brown crystals. The precipitate collected by filtration was washed by a small amount of acetone and dried over diphosphorus pentaoxide in a vacuum desiccator to give **3** as dark violet crystals. Yield: 83.9 mg (6.41×10^{-2} mmol); 92.8%. UV–vis (Tris–HCl buffer at pH 7.6); λ_{max}/nm (ε): 400.5 (1.37×10^5), 501.0 (1.03×10^4), 532.0 (4.56×10^3), 605.0 (4.96×10^3), 658.5 (3.23×10^4). ^{1}H NMR (300 MHz, DMSO-d_6): 9.79 (s, 1H, β-H), 9.67 (s, 1H, α-H), 9.06 (s, 1H, δ-H), 8.30 (dd, 1H, $J = 12$ Hz, 18 Hz, 2a-H), 6.48 (dd, 1H, $J = 1.2$ Hz, 17 Hz, 2b-H), 6.22 (dd, $J = 1.5$ Hz, 10 Hz, 2b′-H), 5.36 (m, 2H, γa-CH_2), 5.14 (m, 2H, 6b-CH_2), 4.60 (m, 2H, 7-H, 8-H), 4.51 (m, 2H,γc-CH_2), 4.33 (m, 2H, 7d-CH_2), 4.08 (m, 2H, 6a-CH_2), 3.80 (q, 2H, $J = 7.3$ Hz, 4a-CH_2), 3.63 (s, 3H, 5a-CH_3), 3.55 (m, 4H, 7c-CH_2, γb-CH_2), 3.50 (s, 3H, 1a-CH_3), 3.30 (s, 9H, 6c-CH_3, 6c′-CH_3, 6c″-CH_3), 3.29 (s, 3H, 3a-CH_3), 3.00 (s, 9H, γd-CH_3, γd′-CH_3, γd″-CH_3), 2.84 (s, 9H, 7e-CH_3, 7e′-CH_3, 7e″-CH_3), 2.60–3.01 (m, 2H, 7b-CH_2), 2.17–2.50 (m, 2H, 7a-CH_2), 1.69 (d, 3H, $J = 6.3$ Hz, 8a-CH_3), 1.67 (t, 3H, $J = 7.6$ Hz, 4b-CH_3), −1.33 (br s, 1H, NH), −1.48 (br s, 1H, NH). FABMS (thioglycerol): *m*/*z*; 856 (M+1), 982 (M+I). Anal. Calcd for $C_{49}H_{72}I_3N_7O_6 \cdot 4H_2O$: C, 45.00; H, 6.17; N, 7.50. Found: C, 45.03; H, 6.07; N, 7.44.
15. To 5.52 mg (6.34×10^{-3} mmol) **2** in 1.5 mL anhydrous acetone was added 4.0 mL of iodomethane after replacement of the atmosphere by nitrogen. The reaction mixture was stirred for about 30 h at room temperature to afford brown crystals. The precipitate collected by filtration was washed by a small amount of acetone and dried over diphosphorus pentaoxide in a vacuum desiccator to give **4** as dark violet crystals. Yield: 6.46 mg (4.51×10^{-3} mmol); 78.6%. UV–vis(Tris–HCl buffer at pH 7.6); λ_{max}/nm(ε): 404.5 (9.55×10^4), 503.5 (8.33×10^3), 535.0 (3.63×10^3), 609.0 (4.09×10^3), 663.0 (2.56×10^4). ^{1}H NMR (300 MHz, CD_3OD): 9.56 (s, 1H, β-H); 9.34 (s, 1H, α-H); 9.30 (s, 1H, δ-H); 8.90, 8.83, 8.57 (d, d, d, each 1H, py-4-H); 8.84, 8.66, 7.98 (s, br s, each 1H, py-2-H); 8.37, 8.25, 7.49 (d, d, d, each 1H, py-6-H); 8.12, 7.75, 7.43 (t, t, t, each 1H, py-5-H); 7.96 (m, 1H, 2a-H); 6.27 (dd, 1H, 2b-H); 6.08 (dd, 1H, 2b′-H); 5.88 (m, 2H, γa-CH_2); 5.25 (m, 2H, γb-CH_2); 5.28 (s, 2H, 6a-CH_2); 4.31–4.50 (m, 2H, 7-H, 8-H); 4.43 (s, 3H, 6-Py-NCH_3); 4.03 (s, 3H, γ-Py-NCH_3); 3.85 (s, 3H, 7-Py-NCH_3); 3.49 (q, 2H, 4a-CH_2); 3.41 (s, 3H, 5a-CH_3); 3.31 (s, 3H, 1a-CH_3); 3.11 (s, 3H, 3a-CH_3); 2.81 (s, 2H, 7c-CH_2): 2.44, 2.20 (m, 2H, 7b-CH_2); 2.19–1.97 (m, 2H, 7a-CH_2); 1.62 (d, 3H, 8a-CH_3); 1.61 (t, 3H, 4b-CH_3). FABMS (triethanolamine); *m*/*z*: 915 (M+1), 1041 (M+I). Anal. Calcd for $C_{55}H_{60}I_3N_7O_6 \cdot 7.5H_2O$: C, 46.17; H, 5.28; N, 6.85. Found: C, 45.89; H, 4.97; N, 6.89.
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17. The vis spectral absorbance of **3** and **4** in Tris–HCl buffer at pH 7.6 and 25 °C obeys the Lambert–Beer law up to 2.5×10^{-5} M. At the concentration of this level or above, their vis spectral bands exhibit small blue shift and hypochromisity. In addition, the CD spectral patterns of **3** and **4** are also changed at the concentrations of higher than 2.5×10^{-5} M. Thus, tri-cationic **3** and **4** in the concentration range of $0–2.5 \times 10^{-5}$ M are soluble in Tris–HCl buffer at pH 7.6 and 25 °C as a monomer.