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SYNTHESIS, PHYSICOCHEMICAL PROPERTIES AND PHOTOCYTOTOXICITY OF FIVE NEW δ-SUBSTITUTED CHLORIN E6 DERIVATIVES

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Abstract: Five new δ-substituted chlorin e6 derivatives were synthesized, and their physicochemical properties and photocytotoxic effects on HGC-27 cells were examined. The results indicated that the triplet lifetime of these derivatives played an important role in their photocytotoxicity.

Introduction

Photodynamic therapy (PDT) has been proved useful in the treatment of cancer.[1] The photodynamic effect is exerted by exposure to visible light after selective retention of a photosensitizer in the tumor tissue. Hematoporphyrin derivatives (Hpd), which are obtained by acetylation followed by alkaline hydrolysis of hematoporphyrin,[2] are widely used as photosensitizers.[1] However, Hpd comprise numerous compounds and their active principle is still unknown.[3] In addition, Hpd show only weak absorption at the Q band region (630 nm) though red light (>600 nm) is effective in PDT because of better tissue penetration.[4] New photosensitizers for PDT such as phthalocyanine,[5] purpurin,[6] chlorin e6 (**1a**)[7] and its aspartate derivative[8] have been reported in the last several years. They were all obtained as single substances with strong absorption in the red region (>650 nm).

Because of its abundance in green plants, we chose chlorin e6 (**1a**), a degradation product of chlorophyll a,[9] as a lead compound for generating new agents for PDT. Modification of the porphyrin π-system, such as, insertion of metals or introduction of substituents onto meso positions of

the porphyrin ring, is expected to change drastically the photochemical properties of the compound. Therefore, we synthesized five new δ-substituted chlorin e6 derivatives and examined their physicochemical properties (triplet lifetime, relative fluorescence yield, visible absorption and partition coefficient) and photocytotoxic effects on human gastric cancer cell line (HGC-27) to generate effective agents for PDT.

Results

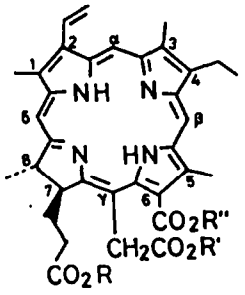
Synthesis of δ-substituted chlorin e6 derivatives.

Since photosensitizers with sufficient water-solubility are required for *in vitro* bioassay and clinical use, we designed five new water-soluble chlorin e6 derivatives having various substituents at the δ-position.

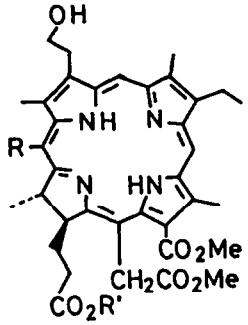
Chlorin e6 trimethyl ester (**1**), the starting compound, was prepared by conventional methods.[10] In advance of the δ-substitution reactions, **1** was transformed into the 2-(2-hydroxyethyl) derivative (**2**) by the method of Smith *et al.*[11a] because protection of the vinyl group at position 2 of **1** was indispensable to avoid some undesirable side reactions.[11a-c] This transformation has also a great advantage to give the derivatives better water-solubility.

After the hydroxyl group at position 2 of **2** was protected by acetylation, substitution reactions at the δ-position were allowed to proceed. Metallation with copper(II) acetate followed by Vilsmeier formylation gave **3b**, which was demetallated to yield the δ-formyl compound (**3**). The δ-methyl compound (**4**) was obtained by the reduction of **3b** with sodium borohydride in acetic acid as described by Smith *et al.*[11a] Chlorination of **2b** was achieved by treatment with 5 % hydrogen peroxide and hydrochloric acid in chloroform. Nitration was carried out with thallium (III) nitrate in tetrahydrofuran.[11b] The structures of these derivatives (**1-6**) were confirmed by the spectral measurements (UV, ^{1}H NMR and EI-MS) and elemental analyses.

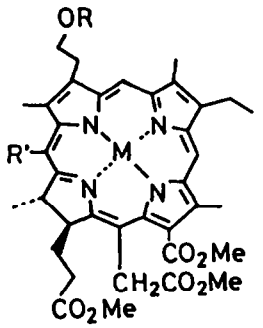
For *in vitro* bioassay, photosensitizers with sufficient water-solubility and adequate hydrophobicity are desirable because cellular uptake of drugs is dominated by their hydrophobicity.[12] Therefore, we partially hydrolized **1**, which has three methyl esters with different reactivity.[13] Acid catalyzed hydrolysis of **1** gave the 6,γ-dimethyl ester (**1b**). Hydrolysis at position 7 of **1** was confirmed by the disappearance of the methyl signal of **1** at δ 3.62 in the ^{1}H NMR (chloroform-*d*, 400 MHz), which was assigned to the 7-methyl group.[14] Compounds **2-6** were similarly hydrolyzed to give the corresponding 6,γ-dimethyl esters (**2a-6a**). Although **1b** was hardly soluble in water, **2a-6a** had sufficient water-solubility. This indicates that the hydroxyl group at position 2 increases the



R	R'	R"	
CH_3	CH_3	CH_3	1
H	H	H	1a
H	CH_3	CH_3	1b



R	R'	
H	CH_3	2
H	H	2a
CHO	CH_3	3
CHO	H	3a
CH_3	CH_3	4
CH_3	H	4a
Cl	CH_3	5
Cl	H	5a
NO_2	CH_3	6
NO_2	H	6a



R	R'	M	
$COCH_3$	H	2H	2b
$COCH_3$	H	Cu	2c
$COCH_3$	CHO	Cu	3b

water-solubility of the derivative. Chlorin e6 (1a), a potent photosensitizer,[7,8c] was prepared by alkaline treatment of 1, and used as a control compound for in vitro bioassay. Structures of 1a-6a were confirmed by the spectral measurements (UV, ^1H NMR and FAB-MS).

Physicochemical properties of the δ-substituted chlorin e6 derivatives.

Table 1 shows the visible absorption, fluorescence, relative fluorescence yield and triplet lifetime of 1a-6a in two aquatic media. In the absorption spectra (Q band), the δ-formyl derivative (3a) showed a significant red shift (λ_{max} nm: 694): the other δ-substituted derivatives (4a-6a) showed a smaller red shift than 3a, and had almost the same visible absorption spectra. However, the emission properties of these compounds were quite different. Substitution with the electron-withdrawing groups (CHO, Cl and NO_2) drastically lowered the fluorescence yield and triplet lifetime.

Table 2 shows the logarithms of the partition coefficients between lecithin bilayered liposome and water [log P (L/W)] of 1a-6a. The 6,γ-dimethyl esters (2a-6a) proved to have similar hydrophobicity, while the tri-carboxylic acid (1a) was about 10-times more hydrophilic than these compounds.

Table 1 Photochemical properties of **2a-6a** in the aquatic media

Compound	Visible absorption[a] (Soret band) λ_{max} (nm)	(Q band) (nm)	Fluorescence[a,b] (emission) λ_{max} (nm)	Relative fluorescence yield	Triplet lifetime[c] (ms)
1a	402	650	660	0.6	1.3
2a	395	650	652	1.0	0.8
3a	400	694	700	0.02	<0.2
4a	405	660	665	0.6	1.3
5a	395	666	665[d]	0.02	<0.2
6a	396	670	663[d]	0.004	<0.2

[a]Measured in water containing 0.9 % sodium chloride. [b]Excited at the wavelength of each excitation maximum. [c]Determined by measurement of the delayed fluorescence in water containing 0.9 % sodium chloride and 1 % bovine serum albumin.[15] [d]Could not be determined with certainty because of their quite weak fluorescent intensity.

Table 2 Logarithms of the partition coefficients between lecithin bilayered liposome and water of **1a-6a**

Compound	log P (L/W)
1a	3.20
2a	4.23
3a	4.00
4a	4.52
5a	3.98
6a	4.32

Table 3 ID_{50} Values of inhibition of the cell growth by **1a-6a**

Compound	Inhibition of the cell growth: with irradiation log $1/ID_{50}$ (log 1/M)	without irradiation log $1/ID_{50}$ (log 1/M)
1a	5.88	4.97
2a	7.28	6.24
3a	5.85	5.69
4a	7.14	6.19
5a	6.36	6.10
6a	6.64	5.98

In vitro photocytotoxicity of the δ-substituted chlorin e6 derivatives.

Fig. 1 shows the photocytotoxic effects of the δ-substituted chlorin e6 derivatives on HGC-27 cells. Inhibition of cell growth (%) is plotted against the drug concentration. In this assay, irradiation was carried out with light of wavelengths longer than 600 nm obtained by passing the light from a 150 W halogen lamp through a cut-off filter. Since photocytotoxicity of the drug was markedly lowered in the presence of serum, the cells were incubated with the drugs in serum-free medium. This was probably caused by the partition of the drug into a lipophilic fraction of the serum such as a lipoprotein.[16] In this assay, cell growth was not inhibited during the incubation in the serum-free medium and the irradiation did not have any hyperthermic effect. No metabolite was

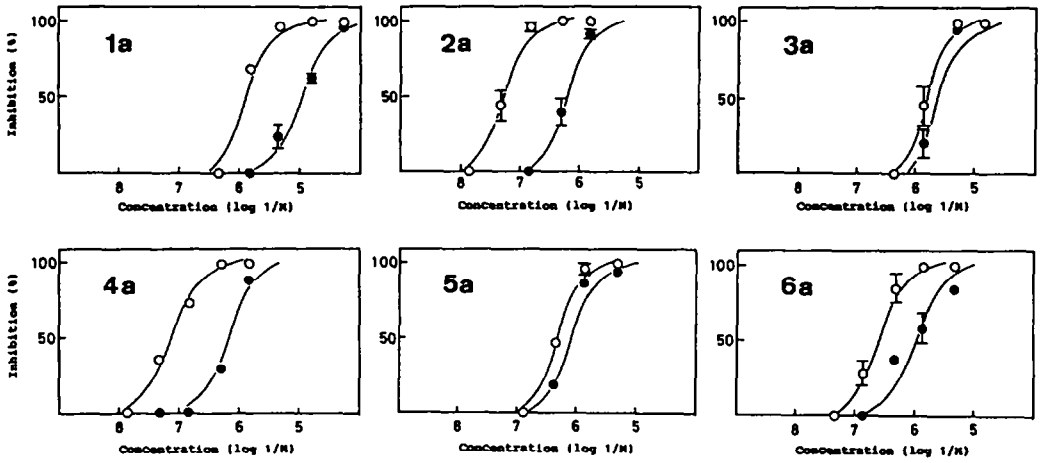


Fig. 1 Photocytotoxicity on HGC-27 cells by δ-substituted chlorin e6 derivatives. Inhibition of the cell growth (%) is plotted against the concentration of each drug. Each point is the average ±SD of triplicate measurements. ○ with irradiation, ● without irradiation.

detected during 30 min incubation by HPLC analysis (not shown).

Table 3 shows the ID_{50} values for inhibition of cell growth by the δ-substituted chlorin e6 derivatives. Compounds **2a** and **4a** proved to be potently photocytotoxic (ID_{50}: 34 and 48 ng/ml, respectively). On the other hand, **3a**, **5a** and **6a** with lower fluorescence yield and shorter triplet lifetime had low photocytotoxicity: irradiation had little effect on the ID_{50} values.

Compound **1a** with one order smaller hydrophobicity than the above five compounds showed significant photocytotoxicity (ID_{50}: 786 ng/ml).

Discussion

PDT using Hpd has several limitations. During the last several years many photosensitizers have been synthesized to obtain more practical agents for PDT. We selected chlorin e6 as the lead compound to synthesize five new δ-substituted chlorin e6 derivatives and investigated the relationship between physicochemical properties and photocytotoxicity on HGC-27 cells.

Substitution at the δ-position of the chlorin ring markedly changed the photochemical properties and photocytotoxicity of the compound. Introduction of the electron-withdrawing groups (CHO, NO_2 and Cl) resulted in a marked decrease in the fluorescence yield and triplet lifetime as well as the photocytotoxicity; introduction of the electron-donating group (CH_3) hardly changed the fluorescence yield and triplet lifetime, and resulted in strong photocytotoxicity (Table 1 and Fig. 1). The five derivatives

(2a-6a) had a similar partition coefficient between lecithin bilayered liposome and water (Table 2), which suggests that the cellular uptake of these drugs is similar. It is generally accepted that the photoreaction of porphyrins in PDT occurs via their lowest triplet states [1,17,18] and that the increase of the fluorescence yield results in the decrease of the triplet yield. Therefore, the above results indicate that the triplet lifetime of photosensitizers plays an important role in the photocytotoxic effects on HGC-27 cells. Hitherto, photosensitizers with strong absorption in the red region (>600 nm) have been thought to be effective in PDT[1,18] because of better tissue penetration.[4] Indeed the marked red shift in the Q band (λ_{max} nm: 694) of 3a may have enhanced the tissue penetration of light, but 3a had low photocytotoxicity because of its extreamly short triplet lifetime. This indicates that triplet lifetime, not strong absorption in the red region, must be taken into account first in the synthesis of effective agents for PDT.

Recently Takemura et al. have also demonstrated the efficiency of photodynamic action in biological systems was governed essentially by the triplet lifetime of the photosensitizers using P388 cells and diethylene triamine pentaaceticacid ester of 4-[1-(2-hydroxy-ethyloxy)ethyl]-2-vinyl-deuteroporphyrin-IX gallium (III), zinc (II) or manganese (III) complex.[17] The present work coincides well with this result.

Cell membrane permeability of the drugs could also influence their in vitro photocytotoxicity.[19,20] Compound **1a** with photochemical properties similar to those of **2a** and **4a** was about 10-fold less active than **2a** and **4a** both in photocytotoxicity and cytotoxicity without irradiation (Fig. 1). This is attributed to the difference in the cellular uptake of the drug.[12] Compound **2a** and **4a** might be more effective PDT agents than chlorin e6 (**1a**).

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Experimental

General remarks.

Melting points are not corrected. The following spectroscopic and analytical instruments were used: UV, Shimadzu UV-200; ^{1}H NMR, JEOL GX400 (400 MHz, ref. TMS) and Varian VXR200 (200 MHz, ref. TMS); MS, JEOL JMS-DX300 (EI-MS: 70 eV, 300 μA; FAB-MS: Ar, m-nitrobenzylalcohol as a matrix); fluorescence spectra, Shimadzu RF-503A; HPLC, Waters Model 600E with a UV detector. Spectrophosphorimeter for determination of triplet lifetime was described in ref. 15) and 17).

HPLC was carried out on a TSK ODS-120T column (Toso Co. Ltd.) for analytical use and μ-Bondasphere C_{18} (15 μ) column (Waters Associates) for preparative use. Wako C-100 gel (silica gel, Wako pure Chemical Industries) deactivated with 20% H_2O (Brockmann grade V) before use, alumina neutral (70-230 mesh, E. Merck) deactivated with 15% H_2O (Brockmann grade V) before use, and YMC I-40/64 gel (ODS, Yamamura Chemical Laboratory) were used for column chromatography.

Pheophorbide a (PPBa) was purchased from Toyo-Hakka Co. Ltd. Methylpheophorbide a (Me-PPBa) obtained from PPBa by methylation with 5% H_2SO_4 in MeOH was purified by column chromatography on the alumina neutral using CH_2Cl_2 and submitted for the starting material of the δ-substituted derivatives.

Chlorin e6 trimethyl ester (1).

Compound **1** was obtained from Me-PPBa by the method of Smith et al.[10] with some modifications. The spectral data (UV, ^1H NMR and EI-MS), melting point and elemental analysis of **1** coincided with those reported previously.[10]

2-(2-Hydroxyethyl)-2-devinylchlorin e6 trimethyl ester (2).

Compound **2** was obtained from **1** by the method of Smith et al.[11a] with some modifications. The spectral data (UV, ^1H NMR and EI-MS), melting point and elemental analysis of **2** coincided with those reported previously.[11a]

2-(2-Acetoxyethyl)-2-devinylchlorin e6 trimethyl ester (2b).

Compound **2** was treated with Ac_2O and pyridine for one night to give **2b** quantitatively. Compound **2b**. ^1H NMR δ ($CDCl_3$) ppm: 1.71 (3H, t, J=7.3Hz), 1.73 (3H, d, J=7.3Hz), 1.75-2.56 (4H, m), 2.06 (3H, s, 2-OAc), 3.31 (3H, s), 3.39 (3H, s), 3.57 (3H, s), 3.63 (3H, s), 3.76 (3H, s), 3.78 (2H, q, J=7.3Hz), 4.20 (2H, t, J=7.3Hz), 4.25 (3H, s), 4.45-4.37 (2H, m), 4.77 (2H, t, J=7.3Hz), 5.22 (1H, d, J=18.6Hz), 5.34 (1H, d, J=18.6Hz), 8.69 (1H, s, δ-H), 9.44 (1H, s), 9.69 (1H, s). EI-MS m/z (%): 698 (M^+, 100). Anal. Calc. for $C_{39}H_{46}N_4O_8$: C, 67.03; H, 6.63; N, 8.01. Found: C, 66.68; H, 6.65; N, 7.94 %.

δ-Formyl-2-(2-hydroxyethyl)-2-devinylchlorin e6 trimethyl ester (3).

To the CH_2Cl_2 solution of **2b** (575 mg), $Cu(OAc)_2$-saturated MeOH solution was added until the complete formation of the copper complex. The mixture was evaporated to dryness, and purified by column chromatography on the alumina neutral using $CHCl_3$ to give **2c** quantitatively. To the $ClCH_2CH_2Cl$ solution of **2c**, Vilsmeier complex obtained by DMF (2 ml) and $POCl_3$ (1.8 ml) was added. The mixture was stirred at 50 °C for 1 hr. After addition of aqueous saturated NaOAc (200 ml), it was stirred further for 1 hr at 60 °C. Then it was diluted with $CHCl_3$, and the organic layer was washed with water, dried over Na_2SO_4, and evaporated to dryness to give **3b**, which was used in the next reaction without isolation.

Compound **3b** was dissolved in conc. H_2SO_4 (240 ml) and stirred for 15 min in the dark. The mixture was poured in a mixture of ice (500 g) and NH_4OAc (500 g). After extraction with $CHCl_3$, the organic layer was washed with water, dried over Na_2SO_4, and evaporated to dryness. The residue was treated with 5% H_2SO_4 in MeOH for one night in the dark. After usual work up, purification was achieved by column chromatography on the Wako C-100 gel using toluene containing increasing amounts of acetone to yield **3** (87 mg), which was recrystallized from CH_2Cl_2-MeOH. Compound **3**, 15 % yield from **2**, mp. 119-120 °C. ^1H NMR δ ($CDCl_3$) ppm: 1.46 (3H, d, J=7.0Hz), 1.58-2.64 (4H, m), 1.63 (3H, t, J=7.6Hz), 3.15 (3H, s), 3.22 (3H, s), 3.38 (3H, s), 3.50-4.70 (5H, m), 3.65 (3H, s), 3.74 (2H, q, J=7.6Hz), 3.81 (3H, s), 4.21 (3H, s), 4.90 (1H, d, J=18.7Hz), 5.05 (1H, d, J=18.7Hz), 5.20 (1H, m), 9.30 (1H, s), 9.33 (1H, s), 11.66 (1H, s, δ-CHO). UV λ_{max} ($CHCl_3$) nm (ε): 414 (112,000), 510 (9400), 547 (12,800), 694 (38,800). EI-MS m/z (%): 684 (M^+, 32), 653 (24), 611 (100). Anal. Calc. for $C_{38}H_{44}N_4O_8$: C, 66.22; H, 6.45; N, 8.13. Found: C, 65.94; H, 6.33; N, 7.96 %.

δ-Methyl-2-(2-hydroxyethyl)-2-devinylchlorin e6 trimethyl ester (4).

Compound **3b** (390 mg) dissolved in AcOH (10 ml) was added to the solution of $NaBH_4$ (300 mg) in AcOH (40 ml) at 10 °C. After 10 min, the reaction mixture was poured into aqueous saturated NaCl, and extracted with CH_2Cl_2. The organic layer was washed with aqueous saturated NaCl and aqueous $NaHCO_3$, respectively, dried over Na_2SO_4, and evaporated to dryness. The residue was dissolved in a mixture of TFA (50 ml) and H_2SO_4 (0.5 ml), and

stirred for 30 min in the dark. The mixture was poured into a mixture of ice and NH_4OAc, and extracted with $CHCl_3$. The organic layer was washed with aqueous saturated NaCl and aqueous $NaHCO_3$, respectively, dried over Na_2SO_4, and evaporated to dryness. Treatment with 5% H_2SO_4 in MeOH followed by purification on the Wako C-100 gel using toluene containing increasing amounts of acetone, and on the alumina neutral using CH_2Cl_2 gave **4** (33 mg) as an amorphous powder (21 % yield from **2**). Compound **4**. ^1H NMR δ ($CDCl_3$) ppm: 1.54 (3H, d, J=6.7Hz), 1.60 (3H, t, J=7.6Hz), 1.60-2.57 (4H, m), 3.29 (3H, s), 3.45 (3H, s), 3.53 (3H, s), 3.61 (3H, s), 3.76 (2H, q, J=7.6Hz), 3.83 (3H, s), 3.84 (3H, s), 4.13-4.31 (5H, m), 4.24 (3H, s), 4.57 (1H, q, J=6.7Hz), 4.98 (1H, d, J=18.9Hz), 5.27 (1H, d, J=18.9Hz), 9.43 (1H, s), 9.54 (1H, s). UV λ_{max} ($CHCl_3$) nm (ε): 406 (132,000), 508 (10,000), 538 (6700), 666 (37,800). EI-MS m/z (%): 670 (M^+, 12), 611 (6). Anal. Calc. for $C_{38}H_{46}N_4O_7$: C, 68.04; H, 6.91; N, 8.35. Found: C, 67.93; H, 7.01; N, 8.17 %.

δ-Chloro-2-(2-hydroxyethyl)-2-devinylchlorin e6 trimethyl ester (5).

Conc. HCl (10 drops) and 5% H_2O_2 (5 drops) were added to the $CHCl_3$ solution of **2b** (100 mg), and the mixture was stirred vigorously for 4.5 hr. The reaction mixture was diluted with $CHCl_3$, washed with aqueous saturated NaCl, dried over Na_2SO_4, and evaporated to dryness. After treatment with 5% H_2SO_4 in MeOH, purification by column chromatography on the Wako C-100 gel using toluene containing increasing amounts of acetone gave **5** (44 mg) as an amorphous powder (40 % yield from **2**). Compound **5**. ^1H NMR δ ($CDCl_3$) ppm: 1.65 (3H, d, J=6.8Hz), 1.69 (3H, t, J=7.8Hz), 1.59-2.59 (4H, m), 3.28 (3H, s), 3.54 (3H, s), 3.56 (3H, s), 3.63 (3H, s), 3.73 (2H, q, J=7.8Hz), 3.83 (3H, s), 4.16-4.32 (5H, m), 4.25 (3H, s), 4.87 (1H, q, J=6.8Hz), 5.09 (1H, d, J=19.0Hz), 5.31 (1H, d, J=19.0Hz), 9.52 (1H, s), 9.60 (1H, s). UV λ_{max} ($CHCl_3$) nm (ε) : 404 (134,000), 507 (13,800), 536 (10,000), 612 (6000), 667 (51,000). EI-MS m/z (%): 690 (M^+, 100), 656 (26), 631 (32), 617 (24). Anal. Calc. for $C_{37}H_{43}N_4O_7Cl$: C, 64.29; H, 6.27; N, 8.11. Found: C, 64.29; H, 6.28; N, 7.89 %.

δ-Nitro-2-(2-hydroxyethyl)-2-devinylchlorin e6 trimethyl ester (6).

A THF solution of **2b** (330 mg) and $Tl(NO_3)_3 \cdot 3H_2O$ (300 mg) was heated at 60 °C for 5 min. The mixture was cooled and then bubbled with SO_2(g) for 2 min. After dilution with CH_2Cl_2 and addition of conc. HCl (10 drops), the mixture was poured into water. The organic layer was washed with aqueous $NaHCO_3$ and water, respectively, dried over Na_2SO_4, and evaporated to dryness. After treatment with 5% H_2SO_4 in MeOH, purification was carried out by column chromatography on the Wako C-100 gel using toluene containing increasing amounts of acetone, followed by preparative HPLC using μ-Bondasphere C_{18} (15 μ) with 90% MeOH in water to give **6** (45 mg) as an amorphous powder (13 % yield from **2**). Compound **6**. ^1H NMR δ ($CDCl_3$) ppm: 1.54 (3H, d, J=6.9Hz), 1.69 (3H, t, J=7.6Hz), 1.75-2.61 (4H, m), 3.17 (3H, s), 3.27 (3H, s), 3.54 (3H, s), 3.62 (3H, s), 3.73 (2H, q, J=7.6Hz), 3.83 (3H, s), 4.14-4.39 (5H, m), 4.26 (3H, s), 4.63 (1H, q, J=6.9Hz), 5.10 (1H, d, J=18.8Hz), 5.31 (1H, d, J=18.8Hz), 9.64 (1H, s), 9.66 (1H, s). UV λ_{max} ($CHCl_3$) nm (ε): 398 (120,000), 500 (11,000), 531 (6000), 666 (46,600). EI-MS m/z (%): 701 (M^+, 100), 684 (29), 656 (44). Anal. Calc. for $C_{37}H_{43}N_5O_9$: C, 63.33; H, 6.18; N, 9.98. Found: C, 63.13; H, 6.19; N, 9.75 %.

Chlorin e6 (**1a**).

Compound **1** (200 mg) dissolved in THF (2 ml) was added to 0.5 N KOH-EtOH (200 ml). After stirring for 3 hr in the dark, the mixture was diluted with $CHCl_3$, washed with aqueous citrate, dried over Na_2SO_4, and evaporated to dryness. The residue was chromatographed on YMC I-40/64 gel with 60% CH_3CN in water to give **1a** (83 mg, 42 % yield). Compound **1a**. ^1H NMR δ [$(CD_3)_2CO$] ppm: 1.70 (3H, t, J=7.0Hz), 1.77 (3H, d, J=7.4Hz), 1.78-3.10 (4H, m), 3.28 (3H, s), 3.52 (3H, s), 3.65 (3H, s), 3.90 (2H, q, J=7.0Hz), 4.38-4.76 (2H, m), 5.45 (1H, d, J=16.4Hz), 5.64 (1H, d, J=6.4Hz), 6.13-6.44 (2H, m), 8.22 (1H, m), 9.10 (1H, s), 9.65 (1H, s), 9.84 (1H, s). UV λ_{max} [$(CH_3)_2CO$] nm (ε): 400 (127,000), 499 (9000), 528 (4600), 608 (4500), 664 (11,000). FAB-MS m/z: 597 (MH^+).

Partial acid hydrolysis of **2-6**.

The trimethyl ester compound (**2**, **3**, **4**, **5** and **6**; ca. 50 mg) was dissolved in 50% H_2SO_4 and stirred for 1 hr in the dark. The mixture was poured into aqueous $NaHCO_3$ and extracted with $CHCl_3$. The extract was washed with aqueous saturated NaCl, dried over Na_2SO_4, and evaporated to dryness. Purification by column chromatography on the Wako

C-100 gel using toluene containing increasing amounts of acetone gave the corresponding derivatives as follows:

2-(2-Hydroxyethyl)-2-devinylchlorin e6 6,γ-dimethyl ester (2a). ^{1}H NMR δ ($CDCl_3$) ppm: 1.69 (3H, t, J=7.8Hz), 1.72 (3H, d, J=7.3Hz), 1.73-2.57 (4H, m), 3.27 (3H, s), 3.36 (3H, s), 3.56 (3H, s), 3.72 (3H, s), 3.76 (2H, q, J=7.8Hz), 4.09 (2H, t, J=6.8Hz), 4.22 (3H, s), 4.31 (2H, t, J=6.8Hz), 4.41 (2H, m), 5.23 (1H, d, J=18.8Hz), 5.29 (1H, d, J=18.8Hz), 8.68 (1H, s), 9.38 (1H, s), 9.68 (1H, s). UV λ_{max} ($CHCl_3$) nm (ε): 399 (135,000), 498 (11,000), 526 (3200), 601 (4600), 655 (40,000). FAB-MS m/z: 643 (MH^+).

δ-Formyl-2-(2-hydroxyethyl)-2-devinylchlorin e6 6,γ-dimethyl ester (3a). ^{1}H NMR δ ($CDCl_3$) ppm: 1.43 (3H, d, J=6.8Hz), 1.62 (3H, t, J=7.6Hz), 1.90-2.75 (4H, m) 3.15 (3H, s), 3.18 (3H, s), 3.38 (3H, s), 3.55 (2H, m), 3.78 (3H, s), 3.95 (2H, m), 4.19 (3H, s), 4.23 (2H, q, J=7.6Hz), 4.28 (1H, m), 4.83 (1H, d, J=18.8Hz), 5.20 (1H, m), 5.23 (1H, d, J=18.8Hz), 9.28 (1H, s), 9.32 (1H, s), 11.60 (1H, s). UV λ_{max} ($CHCl_3$) nm (ε): 413 (118,000), 512 (6900), 547 (9500), 641 (5800), 694 (33,000). FAB-MS m/z: 670 (MH^+).

δ-Methyl-2-(2-hydroxyethyl)-2-devinylchlorin e6 6,γ-dimethyl ester (4a). ^{1}H NMR δ ($CDCl_3$) ppm: 1.52 (3H, d, J=6.7Hz), 1.64 (3H, t, J=7.6Hz), 1.70-2.51 (4H, m), 3.28 (3H, s), 3.42 (3H, s), 3.52 (3H, s), 3.74 (2H, q, J=7.6Hz), 3.78 (3H, s), 3.82 (3H, s), 4.10-4.38 (5H, m), 4.21 (3H, s), 4.55 (1H, m), 5.00 (1H, d, J=16.4Hz), 5.24 (1H, d, J=16.4Hz), 9.43 (1H, s), 9.53 (1H, s). UV λ_{max} ($CHCl_3$) nm (ε): 406 (132,000), 508 (10,000), 538 (6700), 666 (37,800). FAB-MS m/z: 657 (MH^+).

δ-Chloro-2-(2-hydroxyethyl)-2-devinylchlorin e6 6,γ-dimethyl ester (5a). ^{1}H NMR δ ($CDCl_3$) ppm: 1.54-2.63 (4H, m), 1.64 (3H, d, J=6.8Hz), 1.68 (3H, t, J=7.8Hz), 3.27 (3H, s), 3.53 (3H, s), 3.55 (3H, s), 3.73 (2H, q, J=7.8Hz), 3.78 (3H, s), 4.15 (2H, t, J=6.8Hz), 4.22 (3H, s), 4.29 (2H, t, J=6.8Hz), 4.55 (1H, m), 4.89 (1H, q, J=6.8Hz), 5.09 (1H, d, J=19.0Hz), 5.27 (1H, d, J=19.0Hz), 9.52 (1H, s), 9.60 (1H, s). UV λ_{max} ($CHCl_3$) nm (ε): 404 (157,000), 507 (11,700), 535 (8300), 613 (4300), 666 (48,700). FAB-MS m/z: 677 (MH^+).

δ-Nitro-2-(2-hydroxyethyl)-2-devinylchlorin e6 6,γ-dimethyl ester (6a). ^{1}H NMR δ ($CDCl_3$) ppm: 1.53 (3H, d, J=6.8Hz), 1.68 (3H, t, J=7.8Hz), 1.74-2.65 (4H, m), 3.14 (3H, s), 3.24 (3H, s), 3.52 (3H, s), 3.70 (2H, q, J=7.8Hz), 3.77 (3H, s), 4.11 (2H, t, J=7.8Hz), 4.22 (3H, s), 4.29 (2H, t, J=7.8Hz), 4.38 (1H, m), 4.62 (1H, m), 5.09 (1H, d, J=19.3Hz), 5.24 (1H, d, J=19.3Hz), 9.62 (1H, s), 9.65 (1H, s). UV λ_{max} ($CHCl_3$) nm (ε): 399 (125,000), 500 (11,000), 531 (6000), 666 (46,500). FAB-MS m/z: 688 (MH^+).

In vitro photocytotoxicity.

HGC-27 cells (10^4 / ml) were incubated for 2 days at 37 °C in Dulbecco's Modified Eagle Medium (DMEM) plus 10% fetal bovine serum (FBS) in a 3.5 cm diameter Petri dish. After removal of the medium, cells were washed with phosphate buffered saline (PBS(-)) and incubated with serum-free DMEM containing drugs at various concentrations for 30 min. The drug-loaded cells were washed with PBS(-), incubated again in the DMEM plus 10% FBS for a few minutes, and irradiated for 5 min (5.8 mW/cm^2) with light supplied from a cold spot PICL-SX (NIPPON P. I. Co. Ltd.) equipped with a halogen lamp (150 W) and two glass fibre light guides, and passed through a cut-off filter O-58 (Kenko Co. Ltd.) to cut off the light of wavelengths shorter than 600 nm. After incubation for 2 additional days, viable cells were counted with a hemocytometer. Control cells were treated in the same way as above without drugs and/or irradiation. Photocytotoxicity was evaluated by the concentration required to cause 50% cell growth inhibition, ID_{50}, which was calculated by a computer program (SAS) with a probit procedure.[21]

Partition coefficient.

Lecithin liposome was prepared from egg yolk as descibed in ref. 20a) except the gel filtration. Partition coefficient was determined from the magnitude of the difference spectrum at Q band, as described in ref. 20b).

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