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Syntheses and screening tests of new chlorin derivatives as photosensitizer

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Abstract

New chlorin derivatives were synthesized from protoporphyrin IX dimethyl ester (**1**) as a starting material. The tumor-localizing abilities were determined by bathochromic and hyperchromic shift test using albumin and the photosensitivities were evaluated by photooxidation test using dansyl-L-methionine. NOH-P-H (**3**) was the most excellent in the both tests. NOH-P-H (**3**) was conjugated with aspartic acid to evaluate the biodistribution with the time progress by nitrogen pulsed laser spectrofluorometry. The concentration of NOH-P-Asp (**15**) in cancer tissues showed the highest concentration at 6 h after the administration and corresponded to five to seven times in other organs. The concentration in cancer tissues showed a rapid decrease at 24 h after the administration. PDT effect of NOH-P-Asp (**15**) was evaluated using tumor-bearing mice. Remarkable anti-tumor effect was observed. NOH-P-Asp (**15**) was expected to be applicable to a new photosensitizer for PDT.

Keywords: Photodynamic therapy; Photosensitizer; Chlorin

1. Introduction

Photodynamic therapy is performed with the combination of the photosensitizer and laser irradiation. It is applied to the therapy of the superficial cancers or age-related macular degenerations [1,2]. It is pointed out that Photofrin is a representative photosensitizer applied to PDT for cancers, but it is required to avoid exposure of skin and eyes to direct sunlight or bright indoor light for 4 weeks after administration and applied to superficial cancers, so new photosensitizes have been desired [3]. Although red light (>600 nm) is more effective in PDT because of its better tissue penetration, porphyrin derivative has only weak absorption (ε: 5000) in the Q band region (630 nm) [4]. It is essential that the second-generation photosensitizers have strong photosensitivity with the light of more than 600 nm for the application to deep-seated cancers or advanced cancers [5–8]. $SnEt_2$ [8,9], mono-L-aspartyl chlorin e6 [10,11], motexafin lutetium [12,13] and Foscan [14,15] have strong absorption in the light of more than 650 nm. We noticed formylchlorins having strong absorption in nearly 670 nm and tried to investigate a new photosensitizer.

2. Experimental

2.1. Materials

Protoporphyrin IX dimethyl ester (**1**) was purchased from Sigma–Aldrich Japan (Tokyo). *N*,*N*-Dimethylacetamide was purchased from Wako Pure Chemical (Osaka) and distilled prior to use. All other solvents and reagents were purchased from Wako Pure Chemical or Sigma–Aldrich Japan and used

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without further purification. Removal of solvents was performed under reduced pressure.

Photoprotoporphyrin IX dimethyl ester (P-Me, **2**) was obtained by the following procedure: PP-Me (**1**, 20 g) was dissolved in chloroform (4 L) and halogen light (150 W, 100,000 lux) was irradiated for 1 week. The solution was concentrated and purified by silica gel column chromatography (chloroform–MeOH). The eluate with 1% MeOH in chloroform was evaporated to dryness and re-crystallized from chloroform to obtain P-Me (**2**, 3 g, 15%). MS (ESI^+) *m/z*: 623 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3342, 1730, 1717, 1654, 1437, 1343, 1266, 1190, 1153, 1135, 1092, 1068, 720, 672. UV–vis (pyridine) λ_{max} (nm) (ε): 393 (63,000), 439 (94,000), 568 (14,000), 613 (6900), 672 (46,000). Anal. calcd. for $C_{36}H_{38}N_4O_6$: C, 69.44; H, 6.15; N, 9.00. Found: C, 69.48; H, 6.08; N, 8.94.

NOH-P-H (**3**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was dissolved in pyridine (10 mL) and added hydroxylamine hydrochloride (200 mg) to stir for 90 min at a room temperature. The solution was added distilled water (50 mL). The precipitate was filtered and dissolved in tetrahydrofuran (20 mL). The solution was added 0.5 mol/L NaOH in water (30 mL) to stir for 90 min at a room temperature. It was added 5% citric acid in water (20 mL) to precipitate. The precipitate was filtered and dried to obtain NOH-P-H (**3**, 1 g, quantitative). MS (ESI^+) *m/z*: 610 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3312, 1705, 1611, 1383, 1107, 1069, 1045, 912, 833, 675. UV–vis (MeOH) λ_{max} (nm) (ε): 406 (78,000), 507 (7000), 547 (8000), 669 (16,000). Anal. calcd. for $C_{34}H_{35}N_5O_6$: C, 66.98; H, 5.79; N, 11.49. Found: C, 66.92; H, 5.78; N, 11.53.

NOAc-P-H (**4**) was obtained by the following procedure: NOH-P-H (**3**, 1 g) was dissolved in pyridine (10 mL) and added acetic anhydride (10 mL) to stir for 90 min at a room temperature. The solution was added distilled water (200 mL) to precipitate. The precipitate was filtered and dried to give NOAc-P-H (**4**, 1 g, 94%). MS (ESI^+) *m/z*: 652 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3312, 1709, 1626, 1537, 1381, 1227, 1109, 1074, 922, 835, 677. UV–vis (MeOH) λ_{max} (nm) (ε): 403 (82,000), 506 (7000), 548 (6000), 668 (10,000). Anal. calcd. for $C_{36}H_{37}N_5O_7$: C, 66.35; H, 5.72; N, 10.75. Found: C, 66.38; H, 5.75; N, 10.71.

MCZ-P-H (**5**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was treated with the same manner as the synthesis of **3** except using carbazic acid methyl ester (1.5 g) and acetic acid (1 mL) instead of hydroxylamine hydrochloride to obtain MCZ-P-H (**5**, 1 g, 93%). MS (ESI^+) *m/z*: 667 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3327, 1720, 1612, 1541, 1441, 1379, 1248, 1107, 1069, 1045, 989, 912, 835, 702, 675. UV–vis (MeOH) λ_{max} (nm) (ε): 407 (89,000), 507 (7000), 553 (11,000), 672 (22,000). Anal. calcd. for $C_{36}H_{38}N_6O_7$: C, 64.85; H, 5.74; N, 12.60. Found: C, 64.88; H, 5.79; N, 12.52.

SCB-P-H (**6**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was treated with the same manner as the synthesis of **3** except using semicarbazide hydrochloride (1 g) instead of hydroxylamine hydrochloride to obtain SCB-P-H (**6**, 1 g, 96%). MS (ESI^+) *m/z*: 652 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3335, 1701, 1574, 1416, 1150, 1069, 1045, 991, 910, 833, 702, 675. UV–vis (MeOH) λ_{max} (nm) (ε): 420 (162,000), 510 (18,000), 552 (27,000), 614 (9000), 673 (45,000). Anal. calcd. for $C_{35}H_{37}N_7O_6$: C, 64.50; H, 5.72; N, 15.04. Found: C, 64.56; H, 5.77; N, 15.09.

TSCB-P-H (**7**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was treated with the same manner as the synthesis of **3** except using thiosemicarbazide hydrochloride (2 g) instead of hydroxylamine hydrochloride to obtain TSCB-P-H (**7**, 1 g, 93%). MS (ESI^+) *m/z*: 668 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3335, 1709, 1601, 1518, 1441, 1379, 1107, 1069, 1047, 991, 910, 835, 675. UV–vis (MeOH) λ_{max} (nm) (ε): 413 (173,000), 426 (173,000), 498 (20,000), 558 (33,000), 615 (12,000), 674 (59,000). Anal. calcd. for $C_{35}H_{37}N_7O_5S$: C, 62.95; H, 5.58; N, 14.68. Found: C, 62.98; H, 5.52; N, 14.66.

AG-P-H (**8**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was treated with the same manner as the synthesis of **3** except using aminoguanidine hydrochloride (1.2 g) instead of hydroxylamine hydrochloride to obtain AG-P-H (**8**, 1 g, 96%). MS (ESI^+) *m/z*: 651 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3281, 1665, 1603, 1535, 1382, 1015, 934, 837, 677. UV–vis (MeOH) λ_{max} (nm) (ε): 405 (95,000), 500 (8000), 558 (13,000), 613 (5000), 671 (27,000). Anal. calcd. for $C_{35}H_{38}N_8O_5$: C, 64.60; H, 5.89; N, 17.22. Found: C, 64.62; H, 5.92; N, 17.16.

NTH-P-H (**9**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was treated with the same manner as the synthesis of **3** except using nicotinohydrazide (1.2 g) and acetic acid (1 mL) instead of hydroxylamine hydrochloride to obtain NTH-P-H (**9**, 1 g, 87%). MS (ESI^+) *m/z*: 714 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3327, 1713, 1674, 1632, 1547, 1383, 1288, 1165, 1109, 1045, 991, 907, 833, 675. UV–vis (MeOH) λ_{max} (nm) (ε): 411 (103,000), 559 (15,000), 673 (23,000). Anal. calcd. for $C_{40}H_{39}N_7O_6$: C, 67.31; H, 5.51; N, 13.74. Found: C, 67.36; H, 5.47; N, 13.79.

NO_2-P-H (**10**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was treated with the same manner as the synthesis of **3** except using nitromethane (60 mL) and sodium ethoxide (2 g) instead of hydroxylamine hydrochloride to obtain NO_2-P-H (**10**, 1 g, 98%). MS (ESI^+) *m/z*: 638 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3333, 1713, 1626, 1555, 1516, 1385, 1325, 1136, 1107, 1072, 1047, 914, 835, 673. UV–vis (MeOH) λ_{max} (nm) (ε): 400 (136,000), 495 (17,000), 565 (15,000), 608 (11,000), 667 (37,000). Anal. calcd. for $C_{35}H_{35}N_5O_7$: C, 65.92; H, 5.53; N, 10.98. Found: C, 65.98; H, 5.47; N, 10.92.

$MeNO_2$-P-H (**11**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was treated with the same manner as the synthesis of **3** except using nitroethane (60 mL) and sodium ethoxide (2 g) instead of hydroxylamine hydrochloride to obtain $MeNO_2$-P-H (**10**, 1 g, 96%). MS (ESI^+) *m/z*: 652 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3333, 1709, 1657, 1558, 1520, 1385, 1136, 1107, 1074,

1047, 991, 910, 837, 719, 673. UV–vis (MeOH) λ_{max} (nm) (ε): 403 (111,000), 499 (13,000), 563 (16,000), 608 (10,000), 667 (40,000). Anal. calcd. for $C_{36}H_{37}N_5O_7$: C, 66.35; H, 5.72; N, 10.75. Found: C, 66.31; H, 5.78; N, 10.70.

$EtNO_2$-P-H (**12**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was treated with the same manner as the synthesis of **3** except using 1-nitropropane (60 mL) and sodium ethoxide (2 g) instead of hydroxylamine hydrochloride to obtain $EtNO_2$-P-H (**12**, 1 g, 94%). MS (ESI^+) *m/z*: 666 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3335, 1717, 1655, 1558, 1387, 1134, 1107, 1069, 1047, 991, 908, 835, 673. UV–vis (MeOH) λ_{max} (nm) (ε): 402 (129,000), 500 (12,000), 564 (16,000), 608 (10,000), 667 (43,000). Anal. calcd. for $C_{37}H_{39}N_5O_7$: C, 66.75; H, 5.90; N, 10.52. Found: C, 66.79; H, 5.83; N, 10.58.

MCN-P-H (**13**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was treated with the same manner as the synthesis of **3** except using malononitrile (10 mL) instead of hydroxylamine hydrochloride to obtain MCN-P-H (**13**, 1 g, 97%). MS (ESI^+) *m/z*: 643 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3335, 2222, 1717, 1612, 1537, 1383, 1227, 1167, 1132, 1107, 1080, 1047, 989, 912, 835, 675. UV–vis (MeOH) λ_{max} (nm) (ε): 394 (133,000), 485 (39,000), 542 (10,000), 650 (25,000), 695 (30,000). Anal. calcd. for $C_{37}H_{34}N_6O_5$: C, 69.14; H, 5.33; N, 13.08. Found: C, 69.18; H, 5.29; N, 13.02.

BAB-P-H (**14**) was obtained by the following procedure: P-Me (**2**, 1 g, 1.6 mmol) was treated with the same manner as the synthesis of **3** except using barbituric acid (1 g) instead of hydroxylamine hydrochloride to obtain BAB-P-H (**14**, 1 g, 88%). MS (ESI^+) *m/z*: 705 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3335, 1724, 1609, 1533, 1377, 1305, 1225, 1107, 1065, 1047, 907, 833, 793, 675 625, 503. UV–vis (MeOH) λ_{max} (nm) (ε): 394 (31,000), 494 (14,000), 648 (7000), 700 (11,000). Anal. calcd. for $C_{38}H_{36}N_6O_8$: C, 64.76; H, 5.15; N, 11.93. Found: C, 64.72; H, 5.19; N, 11.86.

NOH-P-Asp (**15**) was obtained by the following procedure: NOH-P-H (**3**, 1 g, 1.6 mmol) was dissolved in *N*,*N*-dimethylacetamide (70 mL) and added dicyclohexylamine (0.6 g), L-aspartic acid dimethyl ester hydrochloride (4.5 g) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (5 g) to stir for 180 min at a room temperature. The solution was added distilled water (300 mL) to precipitate. The crude precipitate was dried and re-crystallized from ethyl acetate. The crystal was dissolved in tetrahydrofuran (20 mL) and added 0.5 mol/L NaOH in water (30 mL) to stir for 90 min at a room temperature. The reaction solution was added 5% citric acid in water (20 mL) to precipitate. The precipitate was filtered and dried to obtain NOH-P-Asp (**15**, 1 g, 73%). MS (ESI^+) *m/z*: 840 $[M+H]^+$. IR (KBr) ν (cm^{-1}): 3312, 1720, 1612, 1549, 1402, 1227, 1192, 916, 835, 675, 555. UV–vis (MeOH) λ_{max} (nm) (ε): 409 (109,000), 508 (9000), 548 (11,000), 669 (18,000). Anal. calcd. for $C_{42}H_{45}N_7O_{12}$: C, 60.16; H, 5.40; N, 11.67. Found: C, 60.13; H, 5.48; N, 11.62.

2.2. Instrumentation

UV–vis spectra were obtained on a spectrometer UV-2400PC (Shimadzu) and infrared spectra on a FTIR-8200 (Shimadzu) using KBr pallete method. Mass spectra were obtained on a liquid chromatograph mass spectrometer LCMS QP8000 (Shimadzu) using ESI ion source as an interface. Thin-layer chromatography was carried out with silica gel 60 F_{254} (Merck). Column chromatography was carried out with Wakogel C-200 (Wako). Elemental analyses were carried by a vario EL (Elementar).

2.3. The bathochromic and hyperchromic effects in albumin solution to evaluate tumor-localizing property

Human serum albumin was dissolved in and diluted with saline to prepare 1.8% albumin solution. The chlorin derivatives (1 mg) were dissolved in phosphate buffer (pH 8.0, 1 mL) and diluted with saline to prepare 10 mg/L solution, respectively. The chlorin solution (2 mL) was mixed with 1.8% albumin solution (2 mL) or saline (2 mL) to measure UV–vis spectrum. The bathochromic and hyperchromic effects were observed.

2.4. Photosensitivity test using dansyl-L-methionine

Dansyl-L-methionine (0.01 mmol) and test compounds (100 mmol) were dissolved in 0.1 mol/L sodium phosphate buffer (pH 7.4, 1 mL). Halogen light (150 W, 100,000 lux, 10 min, Nippon P.I. Co. Ltd.) was exposed to the solutions under the stirring. The solution (1 μL) was applied to thin-layer chromatography (TLC) at the every 1 min from the start of irradiation and developed with chloroform–methanol (3:2). Dansyl-L-methionine was employed as a probe substrate. The reaction mixture was subjected to TLC analysis and revealed a new spot (Rf 0.5). The new spot was identified as dansyl-L-methionine sulfoxide. The time periods required for the total disappearance of dansyl-L-methionine were measured.

2.5. Biodistribution of NOH-P-Asp (15)

Syrian golden hamsters were implanted with about 1 mm^3 pieces of nitrosoamine-induced pancreatic cancer. All implantations were performed on the back of them. They were used for testing tumor-localizing property when the major axis of each tumor grew up to 7–10 mm. NOH-P-Asp (**15**, 12.5 mg/kg), which had been dissolved in 0.1 mol/L sodium phosphate buffer (pH 8.0) was intravenously injected into tumor-bearing hamsters. At 6, 12, 24 and 48 h after the injection, the hamsters (five per group) were exsanguinated to death and dissected to collect the cancer tissues and organs. Their tissues were irradiated using nitrogen pulsed laser (wavelength: 337 nm, 2 ns) as an exciting light source and the fluorescent spectra (400–1000 nm) were measured [16]. The concentration of the NOH-P-Asp (**15**) in the organs was determined by the surface fluorescence intensities.

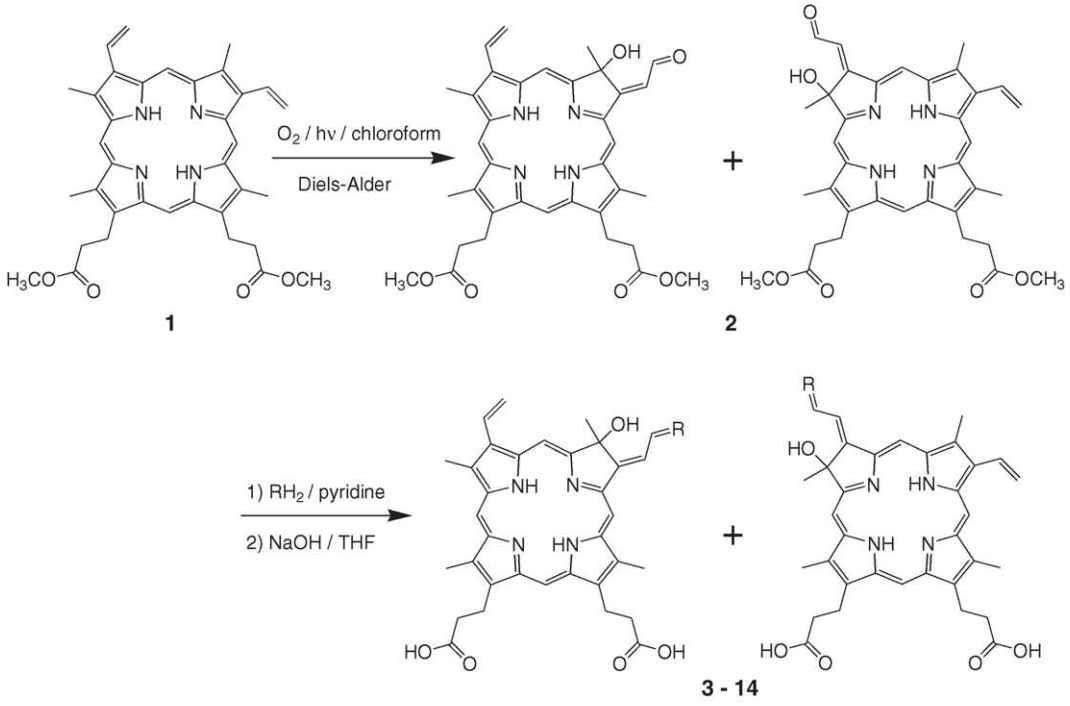
2.6. PDT efficacy

CDF1 mice were implanted with about 1 mm^3 pieces of colon 26 cancer. All implantations were performed on the back of them. They were used for testing in vivo photodynamic effect when the major axis of each tumor grew up to 7–10 mm. NOH-P-Asp (**15**, 10 mg/kg), which had been dissolved in 0.1 mol/L sodium phosphate buffer (pH 8.0) was intravenously injected into tumor-bearing mice ($n = 6$). At 6 h after injection, the tumor surfaces of six mice were irradiated using argon–dye laser ($\lambda = 670$ nm, 220 J/cm^2, 150 mW, Laser Sonics, USA). The long and short diameters (mm) of tumors were measured. The tumor size was calculated as: (long diameter + short diameter)/2.

3. Results

3.1. Syntheses of chlorin derivatives

Protoporphyrin IX dimethyl ester (PP-Me, **1**) was dissolved in chloroform. The solution was irradiated using halogen light to convert formylchlorin derivative (P-Me, **2**) with 50% yield [17,18]. PP-Me (25%) and high polar compounds (25%, not identified) were remained. The photooxidation



Code	Name	R
3	NOH-P-H	NOH
4	NOAc-P-H	$NOCOCH_3$
5	MCZ-P-H	$NNHCOOCH_3$
6	SCB-P-H	$NNHCONH_2$
7	TSCB-P-H	$NNHCSNH_2$
8	AG-P-H	$NNHC(NH_2)=NH_2$
9	NTH-P-H	$NNHCOC_5H_4N$
10	NO2-P-H	$CHNO_2$
11	MeNO2-P-H	$CH(NO)_2CH_3$
12	EtNO2-P-H	$CH(NO_2)CH_2CH_2$
13	MCN-P-H	$C(CN)_2$
14	BAB-P-H	$C(CONH)_2C=O$

Fig. 1. Synthesis scheme of chlorin derivatives.

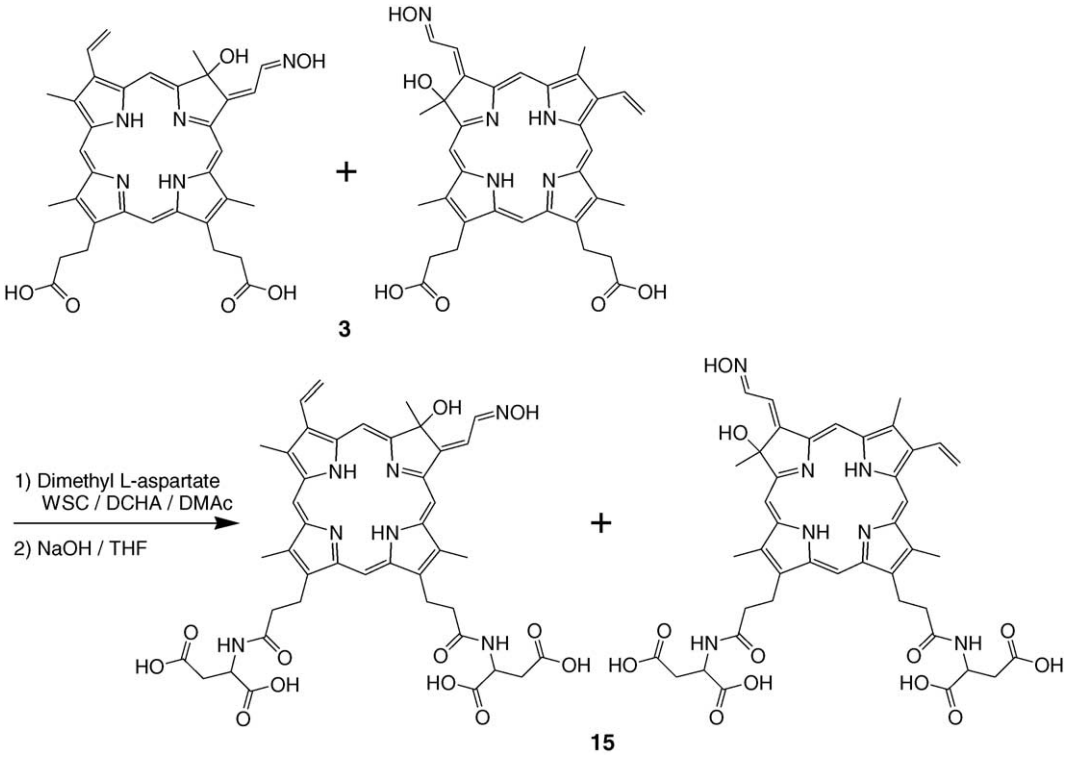


Fig. 2. Synthesis process of NOH-P-Asp (**15**).

reaction yield did not increased even if irradiation conditions were exchanged. The oxidation or degradation rate of P-Me (**2**) was considered to be faster than the conversion rate of PP-Me (**1**) to P-Me (**2**). The solution was concentrated and purified by a silica gel chromatography to remove PP-Me (**1**) and the high polar compounds. The following re-crystallization from chloroform gave P-Me (purity 98% or more). The yield was 30% from PP-Me (**1**). Aldehyde group in P-Me (**2**) was quantitatively reacted with hydradine or activated methylene group. The chemical bond made in the previous step was stable during the next hydrolysis to obtain desired compound (**3**–**14**, Fig. 1) quantitatively. NOH-P-H (**3**) was dissolved in *N*,*N*-dimethylacetamide and added dicyclohexylamine and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride to react with L-aspartic acid dimethyl ester hydrochloride almost quantitatively. The dimethyl aspartate-condensed compound was hydrolyzed to give NOH-P-Asp (**15**, Fig. 2) quantitatively. Thirteen kinds of chlorin derivatives (**3**–**15**, Figs. 1 and 2) were synthesized by above-mentioned process and their chemical structures were identified by the instrumental analyses (MS, IR, UV–vis, element analysis). Solubility in 0.1 mol/L sodium phosphate buffer (pH 8.0) was 10 mg/mL for NOH-P-H (**3**) and was 100 mg/mL for NOH-P-Asp (**15**).

Table 1
Albumin test and photosensitivity test of chlorin derivatives

Code	Name	Not containing albumin		Containing 0.9% of albumin		Albumin test value	Photosensitivity
		λ_{max} (nm)	Intensity	λ_{max} (nm)	Intensity		
3	NOH-P-H	392	0.688	412	0.793	24.0	2
4	NOAc-P-H	380	0.497	401	0.501	21.0	2
5	MCZ-P-H	398	0.658	413	0.721	16.5	2
6	SCB-P-H	400	0.559	416	0.613	17.6	2
7	TSCB-P-H	404	0.497	420	0.574	19.2	2
8	AG-P-H	387	0.257	401	0.334	18.2	10
9	NTH-P-H	400	0.548	416	0.640	19.2	2
10	NO_2-P-H	383	0.600	396	0.642	14.3	3
11	$MeNO_2$-P-H	388	0.528	400	0.571	13.2	3
12	$EtNO_2$-P-H	386	0.504	400	0.547	15.4	3
13	MCN-P-H	392	0.373	405	0.475	16.9	10
14	BAB-P-H	390	0.289	402	0.362	15.6	10
15	NOH-P-Asp	391	0.640	410	0.748	22.8	2

Table 2
Biodistribution test of NOH-P-Asp (**15**)

Time (h)	Relative fluorescence intensity			
	Tumor	Liver	Lung	Kidney
6	2.72	0.54	0.36	0.45
12	2.55	0.58	0.18	0.36
24	1.36	0.26	0.17	0.26
48	0.57	0.09	0.14	0.09

3.2. Bathochromic and hyperchromic effects in albumin solution (albumin test)

The maximum absorption wavelength differences between 0% and 0.9% albumin solutions were found. The absorption intensity ratios of 0–0.9% albumin solution were calculated. The OD_{max} difference value was multiplied by OD_{max} ratio to yield the albumin test value. The value expressed numerically the degree of bathochromic and hyperchromic effects in albumin solution. The test was performed for 12 kinds of chlorin derivatives (**3**–**14**, Fig. 1). As the result, NOH-P-H (**3**) showed the highest albumin test value and hydrazide derivatives (**5**–**9**) had the good performances (Table 1). NOH-P-H (**3**) interacted robustly with albumin in saline; therefore, it was expected to have the excellent tumor-localizing property. NOH-P-H (**3**) was connected with aspartic acid to increase water solubility, but it was feared for deterioration in tumor-localizing property. The albumin test value of NOH-P-Asp (**15**) reduced approximately by 5% in comparison with NOH-P-H (**3**) but it was superior to the other derivatives (**4**–**14**).

3.3. Photosensitivity

Halogen light irradiation was performed to dansyl-L-methionine and test compound dissolved in sodium phosphate buffer. A time course with sampling at 1 min time intervals was performed for quantitative evaluation. The time periods required for the total disappearance of dansyl-L-methionine are summarized in Table 1. These should correspond to photosensitivity. As the result, 10 derivatives (**3**–**7**, **9**–**12** and **15**) showed the high photosensitivity (Table 1).

3.4. Biodistribution

The relative fluorescence intensities are shown in Table 2. NOH-P-Asp (**15**) accumulated with the highest concentration in cancer at 6 h after the administration. The concentration in cancer was five to seven times higher than that in the other organs (Table 2).

3.5. PDT efficacy

The cancer surfaces of six mice in the non-administered group as a control were irradiated using argon–dye laser. In the control group, complete response or reduction of the tumor was not found. NOH-P-Asp (**15**) was intravenously injected to six mice and the cancer surfaces of them were irradiated using argon–dye laser at 6 h after the administration. The general condition of mice after administration of NOH-P-Asp (**15**) was good. As the result, the tumor was disappeared in two cases, reduced the size to one-third in three cases and to one-fourth in one case after 1 week.

4. Discussion

NOH-P-H (**3**) and NOH-P-Asp (**15**) showed excellent performances in the albumin test (Section 3.2) and the photosensitivity test (Section 3.3). NOH-P-Asp (**15**) has the advantage of good water solubility as a drug for injection in comparison with NOH-P-H (**3**). Nearly 670 nm was thought to be effective for PDT using NOH-P-Asp (**15**), because the longest absorption wavelength in 0.9% albumin solution was 671 nm (ε: 25,500). The excitation wavelength of 650–700 nm is effective for PDT [8], because a wide range of wavelength of light is absorbed due to water, hemoglobin and melamine in vivo. Mono-L-aspartyl chlorin e6 [10,11] and Foscan [14,15] have an exciting wavelength of 650–700 nm. NOH-P-Asp (**15**), mono-L-aspartyl chlorin e6 and Foscan are expected to be applicable to deep-seated cancers. The concentration of NOH-P-Asp (**15**) in cancer tissues showed a rapid decrease at 24 h after the administration. The concentrations in the other organs showed the lower values than cancer tissues at 6–48 h. The performance of the rapid decrease in caner tissues and the low concentrations in the other organs of NOH-P-Asp (**15**) are thought to relate its water solubility. NOH-P-Asp (**15**) is expected to excrete rapidly from a body and to shorten restriction period (to avoid exposure of skin and eyes to direct sunlight or bright indoor light) in comparison with the other photosensitizers.

5. Conclusion

NOH-P-Asp (**15**) is promising the second-generation photosensitizer for PDT due to excellent tumor-localizing property, high photosensitivity, ideal excitation wavelength and rapid excretion. The capacities as the photosensitizer of their position isomers should be investigated hereafter.

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