THE STRUCTURES OF MONO-L-ASPARTYL CHLORIN e6 AND ITS RELATED COMPOUNDS

Shuichi Gomi,*[a] Toshio Nishizuka,[a] Osamu Ushiroda,[a] Naoki Uchida,[a] Hirotada Takahashi,[b] and Shinjiro Sumi[b]

[a] Pharmaceutical Research Center, Meiji Seika Kaisha, Ltd. 760, Morooka-cho, Kohoku-ku, Yokohama 222-8567, Japan
[b] Pharmaceutical Technology Laboratory, Meiji Seika Kaisha, Ltd. 788, Kayama, Odawara, Kanagawa 250-0852, Japan

Abstract - Structures of the photosensitizer mono-L-aspartyl chlorin e6 (**1**) and its related compounds were examined by chemical studies and spectral means. It was clarified that L-aspartyl moiety of **1** was attached to acetic acid side chain of chlorin e6, therfore, the structure of **1** was established to be (2*S*,3*S*)-18-carboxy-20-[*N*-(*S*)-1,2-dicarboxyethyl]carbamoylmethyl-13-ethyl-3,7,12,17-tetramethyl-8-vinylchlorin-2-propanoic acid. The thermal degradation product was determined to be an 18-decarboxyl derivative of **1**. In addition, ^{15}N NMR assignment of **1** in DMSO-d_6 solution was confirmed by ^{1}H-^{15}N HMQC and HMBC techniques. In comparison with ^{1}H chemical shifts in D_2O and DMSO-d_6, it was deduced that **1** in water is not present as a free molecule, but as a complex of two or more molecules.

The photosensitizer mono-L-aspartyl chlorin e6 (**1**)[1] is a promising candidate in the photodynamic therapy of neoplastic disease, especially in early stage of lung cancer, and the clinical trials have been progressing.[2-23] Compound (**1**) is a semi-synthetic agent derived from condensation of chlorin e6 (**2**)[24,25] with L-aspartic acid. However, the structural determinations of **1** itself and related compounds have not been reported to date. There are many literatures on the biochemical and clinical studies in which the structure of **1** was depicted as 23-aspartyl chlorin e6 (**4**) without notices on the structural determinations.[2-10] In our present study, it was clarified that the actual structure of **1** was 34-aspartyl chlorin e6, a positional isomer of **4** concerning with the aspartyl side chain on the basis of a detailed NMR analysis of **1** and other two mono-L-aspartyl derivatives of chlorin e6 which were synthesized by general method.[26,27] The abundant studies concerning structure of porphyrins and chlorins have been carried out by chemical means,[28-31] X-Ray crystallography[32-40] and other spectral analysis.[41,42] But their detailed NMR analyses and assignments, especially in aqueous solution, have been little reported, because of a difficulty on measurement due to a peak broadening and a chemical shift change depending upon a concentration below *ca.* 20 mg/mL of the compounds. Therefore, the structure analysis of this kind of compounds should be done very carefully. In this report, we describe the structures of **1** and its thermal degradation product (**3**) on the basis of chemical studies and NMR analysis. The NMR assignments of related compounds including two positional isomers of **1**, 23-aspartyl chlorin e6 (**4**, previously reported structure of **1**) and 32-aspartyl chlorin e6 (**5**), are also reported.

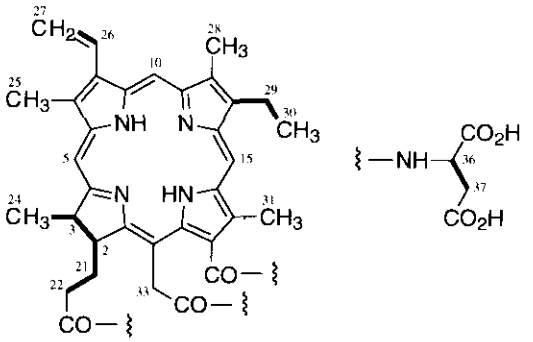
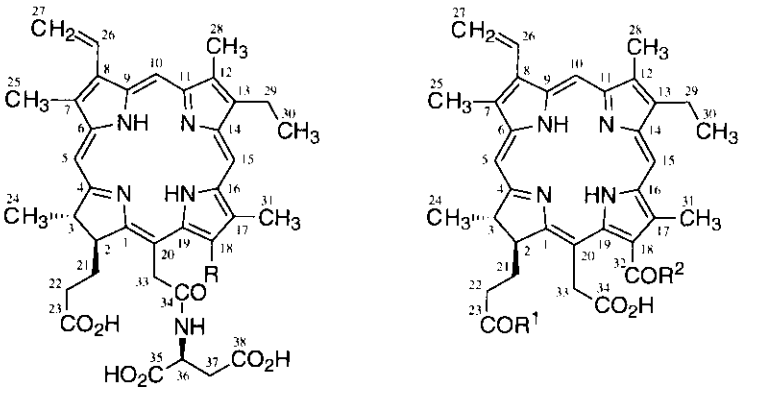


Figure 1. ^{1}H-^{1}H COSY of **1**.



1 : R= $^{32}CO_2H$
3 : R= H

2 : R^1= OH, R^2= OH
4 : R^1= L-Aspartyl, R^2= OH
5 : R^1= OH, R^2= L-Aspartyl

Figure 2. The structures of **1** and the related compounds.

RESULTS AND DISCUSSION

Compound (**1**) is synthesized in good yield by direct condensation of chlorin e6 with L-aspartic acid *via* the active ester with carbodiimide. In order to clarify the structures of the analogs and degradation products of **1**, at first we performed fully examinations of the ^{1}H and ^{13}C NMR of **1** to determine the position of aspartyl group attached to chlorin e6. The molecular formula of **1** was determined to be $C_{38}H_{41}N_5O_9$ (MW 711) by the TSP (thermospray) MS spectrum [*m/z* 712 $(M+H)^+$] and HRFAB (calcd for $C_{38}H_{38}N_5O_9Na_4$ of **1** tetrasodium salt 800.2260, found 800.2246). The NMR analysis of **1** tetrasodium salt in D_2O is described as follows. By the ^{1}H-^{1}H COSY and ^{1}H-^{13}C HSQC (heteronuclear single-quantum coherence)[43] experiments, it was clarified that the sequences of 24-H_3 - 3-H - 2-H - 21-H_2 - 22-H_2 ($J_{2,3}$ < 1 Hz), 26-H - 27-H_2, 29-H_2 - 30-H_3 and 36-H - 37-H_2 as shown by bold lines in Figure 1. Next, the ^{13}C assignment of chlorin e6 moiety was carried out by ^{1}H-^{13}C HMBC (heteronuclear multiple-bond connectivity)[44] technique. The correlation peaks between 30-H_3 (δ 1.20) and C-13 (δ 143.9), between 28-H_3 (δ 0.92) and C-11 (δ 150.8), C-12 (δ 135.4), C-13, and between 29-H_2 (δ 2.46, 2.85) and C-12, C-13, C-14 (δ 148.1) were observed. Therefore, the methyl and ethyl groups were located at C-12 and C-13 on the pyrrole ring, respectively. In the same way, the observation of correlation peaks between 27-H_2 (δ 5.19, 5.28) and C-8 (δ 132.8), between 25-H_3 (δ 2.94) and C-6 (δ 138.0), C-7 (δ 129.9), C-8, and between 26-H (δ 6.52) and

C-7, C-9 (δ 132.3) gave a partial structure of C-6 to C-9 shown in Figure 2. Furthermore, owing to the long-range couplings of 10-H (δ 6.76) and C-8, C-12, the two pyrrole rings mentioned above were connected to each other at C-9 and C-11 through the sp^2 CH group. On the other hand, the correlation peaks between 24-H_3 (δ 1.89) and C-2 (δ 54.5), C-3 (δ 49.6), C-4 (δ 170.5), and between 5-H (δ 9.04) and C-3, C-6, C-7 were observed. Accordingly, C-4 on the dihydropyrrole ring and C-6 on the pyrrole ring were connected through C-5. Moreover, the sequence of C-13 to C-18 was confirmed by the observation of correlation peaks between 31-H_3 (δ 3.67) and C-16, C-17 (δ 131.3*, 134.3*), C-18 (δ 134.0) on a remaining pyrrole ring, between 15-H (δ 9.27) and C-13, C-16, C-17. The isolated methylene protons (δ 5.42, 6.03) at C-33 were coupled to C-19 (δ 135.8), C-20 (δ 103.1), C-34 (δ 175.3) and C-1 (δ 170.7) which was also coupled to 3-H. The remaining carbonyl carbons at δ 183.8 and δ 178.4 were assigned to be C-23 coupled to 22-H_2 (δ 2.85, 3.00) and C-32 having no correlation peak from all protons, respectively. From the above-mentioned result, the main frame including the side chains from C-1 to C-20 of **1** was confirmed. With regard to the aspartyl moiety of **1**, the sequence of C-35 to C-38 was assigned by the observation of the common correlation peaks between 36-H (δ 4.61), 37-H_2 (δ 2.70, 2.75) and C-35, 38 (δ 179.3*, 179.9*). Also, the α-methine (36-H) of aspartic acid was long-range coupled to the carbonyl carbon at C-34 which was strongly coupled to the isolated methylene of 33-H_2. Consequently, the L-aspartyl moiety was attached to acetic acid side chain at C-20 of chlorin e6, and the structure of **1** was determined as shown in Figure 2. Summary of ^1H-^{13}C long-range couplings is represented in Figure 3. The NMR data in D_2O of **1** are listed in Tables 1 and 2. The structure of chlorin e6 derived from chlorophyll a was proposed in 1940[24] and the total synthesis was accomplished in 1960.[25] The stereochemistry of chlorin e6 was reconfirmed by X-Ray analyses of methylpheophorbide a[32,33] and rhodochlorin XV dimethyl ester,[34] which have the same stereochemistry at C-2 and C-3 of chlorin e6.

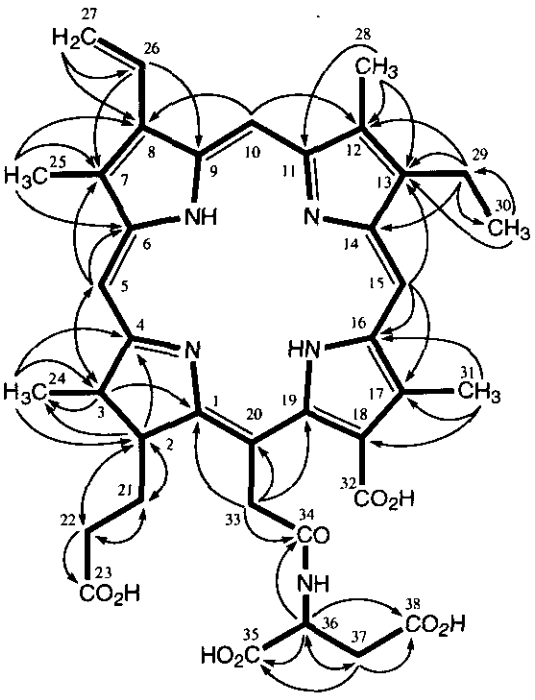


Figure 3. ^1H-^{13}C correlations in the HMBC of **1**.

In order to established strictly the structures of **1**, we synthesized two positional isomers of **1**. The structures of the two isomers were confirmed similarly as the structural determination of **1**. Especially, the attached positions of aspartyl group were certified by the facts described below. In the case of 23-L-aspartyl chlorin e6 (**4**), a correlation peak between an α-methine (36-H, δ 4.55) of aspartyl group and a carbonyl carbon at C-23 (δ 176.4) was observed in the HMBC spectrum, and C-23 was also coupled to 22-H_2 (δ 3.00). In an HMBC spectrum of 32-L-aspartyl chlorin e6 (**5**), it was clarified that three carbonyl carbons at C-23 (δ 183.8), C-32 (δ 171.7) and C-34 (δ 181.6) were coupled to 22-H_2 (δ 2.85, 2.94), an α-methine (36-H, δ 5.17) of aspartyl group and an isolated methylene of 33-H_2 (δ 5.16, 5.58), respectively. By comparison with the ^{13}C chemical shifts of chlorin e6 and those of three aspartyl derivatives, the amide carbons newly formed were shifted upfield by about 7 ppm as shown in Table 2. This supported the structures of three aspartyl derivatives of chlorin e6.

Table 1. ^{1}H NMR data for **1** tetrasodium salt and related compounds in D_2O.

Proton	**1**[a]			**2**[a]			**3**[a]			**4**[a]			**5**[a]		
	δ	M	*J* (Hz)	δ	M	*J* (Hz)	δ	M	*J* (Hz)	δ	M	*J* (Hz)	δ	M	*J* (Hz)
2	4.65	br dd	10.7, 2.0	4.49	br d	10.0	4.68	br dd	10.8, 1.7	4.52	br dd	10.7, 2.0	4.43	br d	11.0
3	4.89	br q	7.3	4.85	*		4.81	*		4.85	br q	7.1	4.80	*	
5	9.04	s		8.99	s		8.82	s		9.11	s		8.87	s	
10	6.76	s		7.06	s		6.91	br s		7.03	s		6.65	br s	
15	9.27	s		9.30	s		8.50	br s		9.29	s		9.23	s	
18	-			-			8.92	s		-			-		
21	2.26	m		2.22	m		2.17	m		2.30	m		2.25	m	
	2.68	m		2.65	m		2.70	m		2.68	m		2.66	m	
22	2.85	m		2.84	m		2.77	m		3.00	m		2.85	m	
	3.00	m		2.95	m		2.94	m					2.94	m	
24	1.89	d	7.3	1.80	d	6.8	1.83	d	7.4	1.81	d	7.1	1.74	d	6.8
25	2.94	s		2.91	s		2.77	br s		3.18	s		2.85	s	
26	6.52	dd	17.8, 11.7	6.59	dd	17.1, 11.5	6.47	br dd	17.0, 11 0	6.84	dd	17.8, 11 7	6.47	dd	17.5, 11.5
27	5.19	d	17.8	5.24	br d	17.1	5.15	d	17.0	5.49	d	17.8	5.19	d	17.5
	5.28	d	11.7	5.28	br d	11.5	5.18	d	11.0	5.51	d	11.7	5.27	d	11.5
28	0.92	s		1.15	br s		1 06	br s		1 07	s		0.91	br s	
29	2.46	dq	14.6, 7.6	2.54	m		2 08	m		2.53	dq	14 9, 7.6	2.43	m	
	2.85	m		3.00	m					3.00	m		2.94	m	
30	1.20	t	7.6	1.24	br t	7.1	0.90	t	7.4	1.23	t	7.6	1.18	br t	7.4
31	3.67	s		3.64	s		3.45	s		3.64	s		3.67	s	
33	5.42	d	18.3	5.24	d	18.6	5.48	d	17 5	5.21	d	18.7	5.16	d	18.6
	6.03	d	18.3	5.85	d	18.6	5.72	d	17.5	5.85	d	18.7	5.58	d	18.6
36	4.61	t	6.3				4.62	dd	7.8, 4.7	4.55	dd	8 3, 4.4	5.17	t	6.8
37	2.70	dd	15.4, 6.3				2.67	dd	15.6, 7.8	2.68	dd	15.4, 8 3	3.02	m	
	2.75	dd	15.4, 6.3				2 74	dd	15.6, 4.7	2.76	dd	15.4, 4.4			

δ : ppm from TSP as an external reference. M : Multiplicity. * : coincided with HDO.
a : The sodium salts of **1** ~ **5** were measured at 20.0, 20.2, 20.2, 20.1, 20.2 mg/0 6 mL, respectively.

Table 2. ^{13}C Chemical shifts for **1** tetrasodium salt and related compounds in D_2O.

Carbon	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	Carbon	**1**[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]
1	170.7	170.7	169.3	170.2	170.0	20	103.1	106.7	103.1	106.8	106.2
2	54.5	54.9	54 5	54.8	54.6	21	33.2	33.0	33 2	31.9	32.9
3	49.6	49.5	49.5	49.6	49.7	22	36 7	36.9	36.4	35.3	36.9
4	170.5	169.7	170.9	169.6	170.6	23	183.8	183.8	183.7	176 4	183.8
5	95.2	95.0	94.3	95 3	94.9	24	24.2	24.2	24.0	24.1	23.9
6	138 0	137.5	138.2	137.7	138.2	25	11.6	11.6	11.5	11.9	11.6
7	129.9	129 6	129.7	129.9	130.1	26	128.5	128.7	128.5	128.9	128.5
8	132.8	132.6	132.8	132.9	132.8	27	121.0	120.8	120.8	121.2	120 9
9	132.3	132.1	132.0	132.1	132.7	28	9 0	9.2	9.0	9.0	8.9
10	96.9	96.8	96.7	96.7	96.3	29	18.8	18.9	18.3	18.9	18.8
11	150.8	150.8	149.9	150.4	151.9	30	17.2	17.2	16.8	17.2	17.2
12	135 4	135 6	134.7	135.5	135.2	31	12.2	12.2	13 4	12 2	11 9
13	143.9	143.9	143.1	143.9	144.2	32	178.4	178.6	-	178.5	171.7
14	148.1	148.3	146.9	148.0	148.2	33	40.1	41.9	40.9	42.0	42.0
15	100.5	99.8	100.1	99 8	100.8	34	175.3	182.1	175.8	182.0	181.6
16	131.3*	131.4*	138.6*	131.4*	130.1*	35	179.3**		179.5**	179.6**	179.1**
17	134.3*	134.2*	132.3*	134.2*	136.8*	36	54 3		54.1	53.9	54.9
18	134.0	134.3	120.2	134.4	128.0	37	41.4		40.8	40.7	40.9
19	135.8	136.0	140.0	136.1	135.8	38	179.9**		179.6**	179.7**	179.8**

δ : ppm from TMS using dioxane (67.4 ppm) as an external reference. *, ** : Interchangeable.
a : The sodium salts of **1** ~ **5** were measured at 20.0, 20.2, 20.2, 20.1, 20.2 mg/0.6 mL, respectively.

Next, we carried out the structural determination of a thermal degradation product (**3**) of **1**. The molecular weight of **3** was determined to be 667 by TSP-MS [*m/z* 668 $(M+H)^+$], which is 44 mass units less than that of **1**. In a ^1H NMR spectrum of **3** sodium salt in D_2O, an additional sp^2 proton (δ 8.92) was observed, and one carbonyl carbon at about δ 178 disappeared on the ^{13}C NMR spectrum. The proton newly formed at δ 8.92 was correlated to C-17 (δ 132.3*), C-19 (δ 140.0) and C-31 (δ 13.4) in the HMBC spectrum. Other long-range ^1H-^{13}C correlation pattern of **3** closely resembled that of **1**. Accordingly, the structure of **3** was determined to be an 18-decarboxyl **1** (34-*L*-aspartyl isochlorin e4) as shown in Figure 2.

By the way, a tetrapyrrole ring skeleton such as chlorins is possible to be several conjugate form. In order to clarify the conjugate form in an aprotic solvent, long-range ^1H-^{13}C couplings of four methyl esters shown in Figure 4 were examined. The NH protons on pyrrole ring of the methyl esters (**6**, **7**, **8** and **9**) resonate around δ -1.4 in $CDCl_3$, which are strongly shielded by the secondary magnetic field due to a magnetically induced ring current in a tetrapyrrole macrocyclic ring.[45] In a ^1H-^{13}C HMBC spectrum of chlorin e6 trimethyl ester (**7**), an NH proton at δ -1.47 was correlated to C-16, C-17 and C-18, so which was assigned to be 16-NH. However, no correlation peak from 6-NH proton was observed in the HMBC spectrum. The HMBC spectra of other methyl esters were much the same as that of **7**. The NMR data in $CDCl_3$ of the methyl esters are listed in Tables 3 and 4.

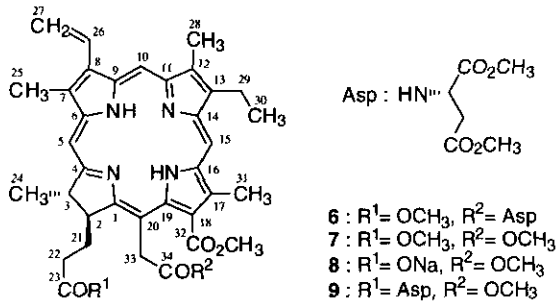


Figure 4 Methyl esters of 1 and the related compounds.

Subsequently, we measured the ^1H-^{15}N HMQC (heteronuclear multi-quantum coherence)[46] and HMBC spectra in order that the ^{15}N chemical shifts of **1** (free acid) in DMSO-d_6 may be assigned. First, by means of ^1H-^{13}C HMBC method, the NH protons at δ -1.69 and -1.97 were assigned to be 6-NH and 16-NH, respectively, because of the observation of clear correlation peaks between 6-NH and C-6, C-7, C-8 and/or C-9, and between 16-NH and C-16, C-18, C-17 and/or C-19. Moreover, an exchangeable proton at δ 8.27 coupled to 36-H (δ 4.60) was assigned to be 34-NH proton. In a ^1H-^{15}N HMQC spectrum, three correlation peaks at δ -245.6, -247.3 and -258.6 for ^{15}N chemical shift were observed, and assigned to be 16-N, 6-N and 34-N (amide), respectively. On one hand, in a ^1H-^{15}N HMBC spectrum, the long-range couplings were observed between 5-H (δ 9.10), 10-H (δ 9.71) and 6-N, and between 15-H (δ 9.79) and 16-N. In addition, two new nitrogen peaks appeared at δ -93.6 (1-N) and -135.8 (11-N), which were coupled to 5-H and 10-H, 15-H, respectively. Accordingly, all nitrogen atoms of **1** in DMSO-d_6 were detected and assigned completely as shown in Table 5. It is reasonable that the aromatic nitrogen (11-N) resonates at *ca.* 110 ppm lower field than the standard pyrrole nitrogens bearing hydrogen (6-N and 16-N), and that the sp^2 nitrogen (1-N) appears further in the lower field by 42.2 ppm from 11-N. The ^{15}N chemical shifts are very important to consider a conjugate form of tetrapyrrole macrocyclic compounds such as chlorins and porphyrins. The result of ^{15}N NMR for **1** also indicates that the conjugate form is an 18 atom path way[41] as shown in Figure 2, which is one of two possible tautomeric forms. It is of interest that the NH protons for **1** and related compounds in an aprotic solvent are localized at the same positions as those of a crystalline state of bacteriochlorophylls.[35,36,40]

The outer protons of the macrocyclic ring in DMSO-d_6 were strongly deshielded due to a magnetically induced ring current,[45] and resonate at δ 9.10 ~ 9.79 for *meso*-protons and at δ 3.30 ~ 3.59 for the methyl

Table 3. ^{1}H NMR data for methyl esters of **1** and related compounds in $CDCl_3$.

Proton	6[a]			7[a]			8[a]			9[a]		
	δ	M	J (Hz)	δ	M	J (Hz)	δ	M	J (Hz)	δ	M	J (Hz)
2	4.49	br d	9.8	4.39	br dd	9.8, 1.7	4.41	br dd	10.3, 2.0	4.48	br dd	9.0,1.7
3	4.45	q	7.3	4.43	q	7.3	4.42	q	7.1	4.45	q	7.3
5	8.74	s		8.72	s		8.72	s		8.74	s	
6-NH	-1.30	br		-1.31	br		nd			-1.30	br	
10	9.53	s		9.52	s		9.52	s		9.53	s	
15	9.67	s		9.66	s		9.66	s		9.67	s	
16-NH	-1.43	br		-1.47	br		-1.48	br		-1.45	br	
21	1.81	m		1.75	m		1.72	m		1.88	m	
	2.29	m		2.20	m		2.17	m		2.27	m	
22	2.26	m		2 18	m		2.22	m		1.69	m	
	2.58	m		2.55	m		2.60	m		2.25	m	
23-OCH_3	3.55	s		3.62	s		-			-		
23-NH	-			-			-			6.18	d	8.3
24	1.72	d	7.3	1.74	d	7.3	1.73	d	7.1	1.76	d	7.3
25	3.44	s		3.44	s		3.43	s		3.46	s	
26	8.01	dd	17.8, 11 5	8.01	dd	17.8, 11.5	8.01	dd	17.8, 11.5	8.03	dd	17.8, 11.5
27	6.11	dd	11.5, 1.5	6.10	dd	11.5, 1.5	6.10	dd	11.5, 1.5	6.11	dd	11.5, 1.5
	6.32	dd	17.8, 1.5	6.31	dd	17.8, 1.5	6.31	dd	17.8, 1.5	6.33	dd	17.8, 1.5
28	3.26	s		3.26	s		3.26	s		3 26	s	
29	3.75	q	7.3	3.74	q	7.7	3.75	q	7.7	3.75	q	7 6
30	1.69	t	7.3	1.69	t	7.7	1.69	t	7.7	1.69	t	7.6
31	3.55	s		3.56	s		3.55	s		3.56	s	
32-OCH_3	4.27	s		4.25	s		4.22	s		4.25	s	
33	5.25	br		5.23	br d	18.8	5.24	br d	18.8	5.30	br s	
				5.34	d	18.8	5.31	d	18.8			
34-OCH_3	-			3.76	s		3.71	s		3.75	s	
34-NH	6.34	br								-		
35-OCH_3	3.08*	br s								3.63*	s	
36	4.82	ddd	8.1, 4.9, 4.4							4.77	ddd	8.1, 4.6, 4 6
37	2.83	dd	16.8, 4.4							2.65	dd	17.2, 4.6
	2.85	dd	16.8, 4.9							2.88	dd	17.2, 4.6
38-OCH_3	3.38*	s								3.51*	s	

δ : ppm from TMS as an internal reference. M : Multiplicity. * : Interchangeable. nd : Not detected.
a : Compounds **6** ~ **9** were measured at 20.2, 20.3, 20.2, 20.1 mg/0.6 mL, respectively.

Table 4. ^{13}C Chemical shifts for methyl esters of **1** and related compounds in $CDCl_3$.

Carbon	6[a]	7[a]	8[a]	9[a]	Carbon	6[a]	7[a]	8[a]	9[a]
1	166.9	166.8	166.7	166.6	23	173.5	173.6	177.8	172.1
2	53.1	52.9	52.9	52.8	24	23.1	22.9	22.9	23.0
3	49.2	49.4	49.3	49.5	25	12.1	12.1	12.1	12 1
4	170 0	169.5	169.4	169.8	26	129.3	129.3	129.3	129.3
5	93.7	93.5	93.5	93.5	27	121.9	121.7	121.7	121.7
6	139.7	139.4	139.5	139.5	28	11.2	11.3	11.3	11.3
7	130.7	130.5	130.5	130.6	29	19.6	19.6	19.6	19.6
8	134.9	134.7	134.8	134.8	30	17.6	17.7	17.6	17.7
9	135.6	135 3	135.4	135.4	31	12.3	12.4	12.4	12.4
10	98.9	98.6	98.7	98.6	32	169.4	169.5	169.4	169.5
11	155.1	154.8	154.8	154.8	33	40.5	38.6	38.6	38.5
12	136.1	135.9	135.9	135.9	34	171.9	173.0	173.2	173.3
13	145.1	145.0	145.0	145.0	35	170.7	-	-	171.0 **
14	149.0	148.9	148.9	148.8	36	48.7	-	-	48.2
15	102.2	102.1	102.1	102 1	37	35.8	-	-	35.9
16	129.3*	129.3*	129.3*	129 3*	38	170.7	-	-	171.4 **
17	136.1*	136.4*	136.4*	136 3*	23-OCH_3	51 6	51.6	-	-
18	123.8	123.3	123.4	123.4	32-OCH_3	53.2	53.0	53.0	53.0
19	135.0	135.4	135.4	135.3	34-OCH_3	-	52.1	52.1	52.1
20	101.7	102.2	102.2	102.4	35-OCH_3	51.3**	-	-	52.7 ***
21	29.6	29.5	29.2	30.2	38-OCH_3	52.3**	-	-	51.9 ***
22	31.2	31.1	30.7	32.4					

δ : ppm from TMS using $CDCl_3$ (77.0 ppm) as an internal reference. *, **, *** . Interchangeable.
a : Compounds **6** ~ **9** were measured at 20.2, 20.1, 20.2, 20.1 mg/0.6 mL, respectively.

Table 5. NMR data for **1** in DMSO-d_6.

Position	^{13}C		^{1}H			^{15}N	Position	^{13}C		^{1}H			^{15}N
	δ	M	δ	M	*J* (Hz)	δ		δ	M	δ	M	*J* (Hz)	δ
1	168.6	s					21	29.9	t	1.71	m		
1-N						-93.6				2.18	m		
2	52.4	d	4.44	br dd	9.5, 2.0		22	31.2	t	2.29	ddd	16.1, 10.0, 4.6	
3	48.2	d	4.58	br q	7.3					2.61	ddd	16.1, 9.3, 5 9	
4	169.6	s					23	174.2	s				
5	94.2	d	9 10	s			24	23.0	q	1.63	d	7.3	
6	138.0	s					25	12.0	q	3.51	s		
6-NH			-1.69	s		-247.3	26	129.3	d	8.29	dd	17.8, 11 6	
7	130.1	s					27	121.9	t	6.16	dd	11.6, 1.4	
8	133.7	s								6.44	dd	17 8, 1.4	
9	133.9	s					28	10.9	q	3.30	s		
10	98.3	d	9.71	s			29	18.9	t	3.81	br q	7.6	
11	153 1	s					30	17.7	q	1.68	t	7.6	
11-N						-135.8	31	12.0	q	3.59	s		
12	136 1	s					32	169.8	s				
13	144.4	s					33	38.9	t	5.17	br d	18.1	
14	148.2	s								5.38	br d	18.1	
15	100.9	d	9.79	s			34	171.1	s				
16	129.1	s					34-NH			8.27	d	9.8	-258.6
16-NH			-1.97	s		-245.6	35	172.4	s				
17	135.4	s					36	48.8	d	4.60	ddd	9.3, 6.7, 5.6	
18	126.4	s					37	36.1	t	2.68	dd	16.6, 5.6	
19	135.3	s								2.78	dd	16.6, 6.7	
20	103.6	s					38	171.8	s				

δ-^{13}C : ppm from TMS using DMSO-d_6 (39.5 ppm) as an internal reference. δ-^{15}N . ppm from $NH_4{}^{15}NO_3$ (0 ppm) as an external reference. M : Multiplicity. Compound **1** was measured at 20.0 mg/0.6 ml.

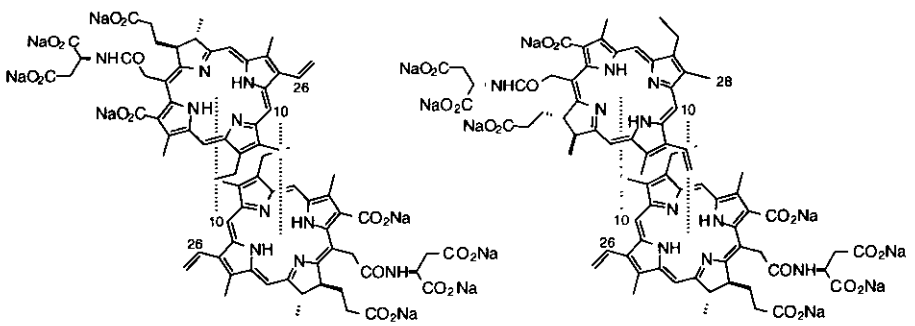


Figure 5. Proposed dimer of **1** tetrasodium salt in water.

protons attached to the β-pyrrole carbon. This evidence suggests that **1** is present as a free molecule in DMSO-d_6 without an interaction with other molecules of **1**. Whereas, in the case of D_2O solution, it is different from this phenomenon. By comparison with the ^{1}H chemical shifts of **1** tetrasodium salt in D_2O and those of the free acid in DMSO-d_6, 10-H, 26-H, 27-H_2, 28-H_3 and 29-H_2 in D_2O appear considerably in the upper field by 2.95, 1.77, 0.97 ~ 1.16, 2.38 and 0.96 ~ 1.35 ppm, respectively, from those in DMSO-d_6. While 21-H_2, one of 22-H_2 and one of 33-H_2 in D_2O resonate slightly in the lower field by 0.50 ~ 0.55, 0.56 and 0.65 ppm, respectively. Especially, 10-H and 28-H_3 in D_2O showed the unusual chemical

shifts at δ 6.76 and 0.92, respectively. These results imply that the molecule of **1** aggregates to form a dimer or a complex of more molecules in D_2O. If the shielding effect to the protons on a quarter molecule from C-8 to C-13 is caused by a macrocyclic ring current of other molecule, the protons might be closely located above the center of other tetrapyrrole ring as shown in Figure 5. This phenomenon has been found in high concentrated chloroform solution of pheophytins and methyl pheophorbides.[47] The molecular packings of chlorophyll derivatives in the crystalline state were reported that neighboring layers are related by π overlap of the pyrrole rings I (C-6 ~ C-9) and III (C-16 ~ C-19) in the different molecules,[33-38] and that adjacent molecules are connected by hydrogen bonds between the hydroxyls of the side chain on the ring I and the keto groups (corresponding to C-32) on the ring V in the bacteriochlorophylls d.[39] The aggregation pattern of **1** in water is deduced to differ from those of related compounds in the crystalline state.

Experimental

General: MS spectra were measured on a JEOL JMS-700 (fast atom bombardment mass spectrometry, FAB-MS) and a Hewlett Packard HP5989A (thermospray mass spectrometry, TSP-MS) mass spectrometers. NMR spectra were recorded on a JEOL JNM-LA400 spectrometer equipped with a field gradient unit using TSP (D_2O) and TMS ($CDCl_3$, DMSO-d_6) for ^1H NMR (400 MHz), and using dioxane (67.4 ppm) in D_2O, $CDCl_3$ (77.0 ppm) and DMSO-d_6 (39.5 ppm) for ^{13}C NMR as a chemical shift standard. ^{15}N chemical shifts were measured by ^1H-^{15}N HMQC and HMBC techniques using $NH_4{}^{15}NO_3$ (0 ppm) as an external reference. All HMBC data were obtained using a mixing time of 60 msec. Digital resolution for ^{15}N chemical shift is 0.48 ppm. Twenty to twenty-one mg/0.6 mL of sample solutions were used for NMR measurement. Chemical shift change is negligible above a concentration of 15 mg/0.6 mL in D_2O. The numbering of **1** skeleton for NMR analysis is used as shown in Figure 2. The NMR data of **1** and the related compounds are shown in Tables 1~5. TLC was done on a silica gel plate [Merck Silicagel 60 F_{254} 5715 (TLC 1), Merck HPTLC 1.05642 Kieselgel 60F (TLC 2)] and a reverse-phase silica gel plate (J.T. Baker C-18, TLC 3). A phosphate buffer for TLC was made with 0.01 M NaH_2PO_4 which was adjusted to pH 6.85 with 0.01 M Na_2HPO_4.

Chemicals: Authentic samples of mono-L-aspartyl chlorin e6 (**1**) tetrasodium salt and chlorin e6 trisodium salt were provided by Porphyrin Products, Inc., Logan, UT, USA. The free acids of these compounds were prepared by acid treatment of the sodium salts. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDAC), dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) were purchased from Sigma Chemical Co. Silica gel (Wakogel C-300, Wako Pure Chemical Industries, Ltd.) and reverse-phase silica gel (DMS DM2035 and ODS DM2035MT, Fuji Silysia Chemical, Ltd.) were used for column chromatography. Other chemicals were purchased from Kokusan Chemical Works, Ltd.

Formation and isolation of the degradation product (3): Compound (**1**) tetrasodium salt (100 mg, 0.125 mmol) was dissolved in water (20 mL), the solution was adjusted to pH 6.7 with 1 M HCl and refluxed at 110℃ for 12 h. After cooling at rt, the reaction mixture was precipitated at pH 2 with 1 M HCl, and the precipitate was collected by centrifugation. The precipitate was suspended in a small amount of water, adjusted to pH 9 with 1 M NaOH, and the solution was applied to a column of DMS gel (200 mL). The column was eluted with 20% aqueous methanol, and the fractions containing a degradation product as detected by TLC were concentrated to give 30.2 mg of crude material. In the same way, the crude sample was further purified by an ODS column chromatography (100 mL) developed with 40% aqueous methanol to afford 21.0 mg (22.9%, powder) of pure degradation product as a sodium salt. The free acid (**3**) was prepared by acid treatment of the sodium salt. [**3**: TSP-MS; *m/z* 668 $(M+H)^+$] The Rf values of **3** and **1** on TLC (TLC 1, chloroform - n-butanol - pyridine - water = 4:4:7:2) were 0.36 and 0.15, respectively.

Compound 1 tetramethyl ester (6): To a suspension of **1** tetrasodium salt (50.0 mg, 0.0625 mmol) in dimethyl sulfoxide (0.45 mL) was added methyl iodide (32 μL, 0.514 mmol) at rt in the dark. The mixture was stirred for 2 h and then poured into water (7.5 mL), and the resultant precipitate was collected by centrifugation. The precipitate was purified by a column of silica gel (10 g) developed with a 40:1 mixture of dichloromethane and methanol to give 27.0 mg (56.2%) of **1** tetramethyl ester as a powder [**6**: FAB-MS; *m/z* 768 $(M+H)^+$, Rf 0.45 (TLC 2, dichloromethane - methanol = 40:1), Rf 0.33 (TLC 3, acetonitrile - phosphate buffer = 9:1)].

Chlorin e6 trimethyl ester (7): To a suspension of chlorin e6 trisodium salt (39.0 mg, 0.0589 mmol) in dimethyl sulfoxide (0.4 mL) was added methyl iodide (28 μL, 0.450 mmol) at rt in the dark. The mixture was stirred for 2 h and then poured into water (7.5 mL), and the resultant precipitate was collected by centrifugation. The precipitate was purified by column chromatography on silica gel (10 g) developed with a 100:1 mixture of dichloromethane and methanol to give 24.0 mg (63.8%) of chlorin e6 trimethyl ester[30,42] as a powder [**7**: FAB-MS; *m/z* 639 $(M+H)^+$, Rf 0.50 (TLC 2, dichloromethane - methanol = 100:1), Rf 0.25 (TLC 3, acetonitrile - phosphate buffer = 9:1)].

Chlorin e6 32,34-dimethyl ester monosodium salt (8): The trimethyl ester (**7**, 75.0 mg, 0.117 mmol) was dissolved in 50% H_2SO_4 (3 mL) at rt in the dark. After stirring for 2 h, the reaction mixture was poured into cold saturated aqueous $NaHCO_3$ (70 mL) and extracted twice with chloroform (30 mL). The extract was washed with brine, dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography on silica gel (20 g) developed with a 15:1 mixture of dichloromethane and methanol to give 50.0 mg (65.9%) of chlorin e6 32,34-dimethyl ester monosodium salt[26] as a powder [**8**: TSP-MS; *m/z* 625 $(M+H)^+$ for free acid, Rf 0.13 (TLC 2, dichloromethane - methanol = 40:1), Rf 0.38 (TLC 3, acetonitrile - phosphate buffer = 9:1)].

23-L-Aspartyl chlorin e6 tetramethyl ester (9): The dimethyl ester monosodium salt (**8**, 39.0 mg, 0.0603 mmol) was treated with 0.1 M hydrochloric acid to convert the corresponding free acid. To a solution of the free acid (37.5 mg) in chloroform (20 mL) was added EDAC hydrochloride (16.0 mg, 0.0835 mmol) and L-aspartic acid dimethyl ester hydrochloride (14.0 mg, 0.0708 mmol) at 0℃ in the dark. After stirring for 2 h, the solution was washed with brine, dried over Na_2SO_4 and concentrated to dryness. The residue was purified by column chromatography on silica gel (20 g) developed with a 40:1 mixture of dichloromethane and methanol to give 28.5 mg (61.5%) of 23-L-aspartyl chlorin e6 tetramethyl ester as a powder [**9**: FAB-MS; *m/z* 768 $(M+H)^+$, Rf 0.48 (TLC 2, dichloromethane - methanol = 40:1), Rf 0.37 (TLC 3, acetonitrile - phosphate buffer = 9:1)].

23-L-Aspartyl chlorin e6 (4): The tetramethyl ester (**9**, 51.0 mg, 0.0664 mmol) dissolved in tetrahydrofuran (1 mL) was added to 0.5 M KOH in ethanol (50 mL). After stirring for 3 h at rt, the mixture was adjusted to pH 2 with 1 M HCl and concentrated to a small volume. The resultant precipitate was collected by centrifugation and washed with water. The crude sample was suspended in a small amount of water, adjusted to pH 9 with 1 M NaOH, and the solution was purified by an ODS column chromatography (100 mL) developed with 40% aqueous methanol to afford 20.5 mg (38.6%) of 23-L-aspartyl chlorin e6 (**4**) tetrasodium salt as a powder [TSP-MS; *m/z* 712 $(M+H)^+$ for free acid, Rf 0.12 (TLC 1, chloroform - n-butanol - pyridine - water = 4:4:7:2)].

Chlorin e6 23,34-dimethyl-32-diphenylmethyl ester (11): To a suspension of chlorin e6 (1.0 g, 1.68 mmol) in acetone (100 mL) was added diphenyldiazomethane (360 mg, 1.85 mmol). The suspension was stirred at rt for 12 h. After removal of the solvent, water (100 mL) and $NaHCO_3$ (700 mg) were added to the residue. The solution was washed with ethyl acetate (100 mL). After the aqueous layer was adjusted

to pH 2.0 with 1 M HCl, the precipitate was collected by centrifugation and washed with water to give 286 mg of crude chlorin e6 32-diphenylmethyl ester [**10**, FAB-MS; *m/z* 763 $(M+H)^+$, Rf 0.46 (TLC 1, chloroform - methanol = 10:1)]. To a solution of the crude sample in a 2:1 mixture of acetone and methanol (15 mL) was added trimethylsilyldiazomethane (10% in hexane, 1.8 mL, 1.58 mmol) and the mixture was stirred under darkness at rt for 12 h. The reaction mixture was concentrated under reduced pressure and purified by silica gel column chromatography (30 g, hexane - ethyl acetate = 5:1) to give 157 mg (11.8%) of **11** as a powder [FAB-MS; *m/z* 791 $(M+H)^+$, Rf 0.26 (TLC 1, hexane - ethyl acetate = 3:1); ^{1}H NMR ($CDCl_3$) δ 4.32 (br dd, *J* = 10.5, 2.2 Hz, 2-H), 4.41 (q, *J* = 7.3 Hz, 3-H), 8.72 (s, 5-H), -1.30 (br, 6-NH), 9.54 (s, 10-H), 9.65 (s, 15-H), -1.46 (br, 16-NH), 1.76 and 2.17 (m, 21-H_2), 2.13 and 2.50 (m, 22-H_2), 3.58 (s, 23-OMe), 1.70 (d, *J* = 7.3 Hz, 24-H_3), 3.45 (s, 25-H_3), 8.05 (dd, *J* = 17.8, 11.5 Hz, 26-H), 6.13 (dd, *J* = 11.5, 1.5 Hz, 27-H), 6.34 (dd, *J* = 17.8, 1.5 Hz, 27-H), 3.28 (s, 28-H_3), 3.75 (q, *J* = 7.6 Hz, 29-H_2), 1.69 (t, *J* = 7.6 Hz, 30-H_3), 3.40 (s, 31-H_3), 5.03 (br d, *J* = 18.8 Hz, 33-H), 5.21 (d, *J* = 18.8 Hz, 33-H), 3.50 (s, 34-OMe), 7.54 (s, C<u>H</u>-Ph_2), 7.66 and 7.70 (m, ortho of CH-<u>Ph</u>$_2$), 7.44 and 7.48 (m, meta of CH-<u>Ph</u>$_2$), 7.37 and 7.40 (m, para of CH-<u>Ph</u>$_2$); ^{13}C NMR ($CDCl_3$) δ 168.8 (s, C-1), 52.9 (d, C-2), 49.3 (d, C-3), 169.5 (s, C-4), 93.5 (d, C-5), 139.4 (s, C-6), 130.5 (s, C-7), 134.7 (s, C-8), 135.4* (s, C-9), 98.6 (d, C-10), 154.8 (s, C-11), 135.9 (s, C-12), 145.0 (s, C-13), 148.9 (s, C-14), 102.1 (d, C-15), 129.3** (s, C-16), 136.3** (s, C-17), 123.2 (s, C-18), 135.7* (s, C-19), 102.3 (s, C-20), 29.6 (t, C-21), 31.1 (t, C-22), 173.5 (s, C-23), 22.9 (q, C-24), 12.1 (q, C-25), 129.4 (d, C-26), 121.7 (t, C-27), 11.3 (q, C-28), 19.6 (t, C-29), 17.7 (q, C-30), 12.5 (q, C-31), 168.0 (s, C-32), 38.4 (t, C-33), 172.9 (s, C-34), 51.6 (q, 23-OMe), 51.9 (q, 34-OMe), 78.8 (d, <u>C</u>H-Ph_2), 127.6 and 127.7 (d, ortho of CH-<u>Ph</u>$_2$), 128.6 and 128.7 (d, meta of CH-<u>Ph</u>$_2$), 128.10 and 128.14 (d, para of CH-<u>Ph</u>$_2$), 128.6 and 128.7 (s, bridge of CH-<u>Ph</u>$_2$), *, ** interchangeable].

Chlorin e6 23,34-dimethyl ester (12): To a solution of **11** (132 mg, 0.167 mmol) in dichloromethane (4 mL) were added anisole (180 μL, 1.66 mmol) and trifluoroacetic acid (1.04 mL, 13.5 mmol) under ice cooling, and the mixture was stirred for 1 h. After concentration of the solvent, the residue was purified by silica gel column chromatography (15 g, dichloromethane - methanol = 20:1) to give 84.0 mg (80.5%) of **12** as a powder [FAB-MS; *m/z* 625 $(M+H)^+$, Rf 0.30 (TLC 1, dichloromethane - methanol = 20:1); ^{1}H NMR ($CDCl_3$) δ 4.40 (br d, *J* = 9.8 Hz, 2-H), 4.44 (q, *J* = 7.1 Hz, 3-H), 8.74 (s, 5-H), -1.36 (br, 6-NH or 16-NH), 9.46 (s, 10-H), 9.60 (s, 15-H), 1.69 and 2.12 (m, 21-H_2), 2.19 and 2.56 (m, 22-H_2), 3.61 (s, 23-OMe), 1.81 (d, *J* = 7.1 Hz, 24-H_3), 3.45 (s, 25-H_3), 8.00 (dd, *J* = 17.8, 11.5 Hz, 26-H), 6.13 (dd, *J* = 11.5, 1.2 Hz, 27-H), 6.32 (dd, *J* = 17.8, 1.2 Hz, 27-H), 3.21 (s, 28-H_3), 3.68 (q, *J* = 7.6 Hz, 29-H_2), 1.64 (t, *J* = 7.6 Hz, 30-H_3), 3.59 (s, 31-H_3), 5.23 (br d, *J* = 18.6 Hz, 33-H), 5.50 (d, *J* = 18.6 Hz, 33-H), 3.82 (s, 34-OMe); ^{13}C NMR ($CDCl_3$) δ 167.8 (s, C-1), 52.9 (d, C-2), 49.6 (d, C-3), 170.3 (s, C-4), 93.9 (d, C-5), 140.1 (s, C-6), 130.9 (s, C-7), 135.1 (s, C-8), 135.6 (s, C-9), 98.5 (d, C-10), 153.8 (s, C-11), 135.6 (s, C-12), 144.9 (s, C-13), 147.7 (s, C-14), 102.3 (d, C-15), 129.4* (s, C-16), 137.5* (s, C-17), 122.4 (s, C-18), 136.4 (s, C-19), 102.5 (s, C-20), 29.3 (t, C-21), 31.2 (t, C-22), 173.6 (s, C-23), 51.7 (q, 23-OMe), 22.8 (q, C-24), 12.1 (q, C-25), 129.2 (d, C-26), 122.1 (t, C-27), 11.2 (q, C-28), 19.6 (t, C-29), 17.5 (q, C-30), 12.7 (q, C-31), 172.3 (s, C-32), 39.1 (t, C-33), 173.7 (s, C-34), 52.4 (q, 34-OMe)].

Active ester of 12 (13): To a solution of **12** (40.0 mg, 0.0640 mmol) in dichloromethane (4 mL) were added 1-hydroxybenzotriazole (21.6 mg, 0.160 mmol) and 4-methylmorpholine (35.2 μL, 0.320 mmol), and then dicyclohexylcarbodiimide (33.2 mg, 0.161 mmol) was added under ice cooling with stirring. After stirring for 13 h, the reaction was quenched by acetic acid. The reaction mixture was diluted with ethyl

acetate (40 mL) and washed with 5% aqueous citric acid, saturated aqueous $NaHCO_3$ and brine. The organic layer was concentrated to dryness, the residue was purified by silica gel column chromatography (5 g, dichloromethane - methanol = 10:1) to give 32.0 mg (67.4%) of **13** as a powder [FAB-MS; *m/z* 742 $(M+H)^+$, Rf 0.44 (TLC 1, hexane - ethyl acetate = 1:1) ; ^{1}H NMR ($CDCl_3$) δ 4.39 (br d, *J* = 9.8 Hz, 2-H), 4.37 (q, *J* = 7.3 Hz, 3-H), 8.57 (s, 5-H), -0.44 (br, 6-NH), 9.34 (s, 10-H), 9.65 (s, 15-H), -0.44 (br, 16-NH), 1.76 and 2.23 (m, 21-H_2), 2.23 and 2.56 (m, 22-H_2), 3.63 (s, 23-OMe), 1.73 (d, *J* = 7.3 Hz, 24-H_3), 3.39 (s, 25-H_3), 7.95 (dd, *J* = 17.8, 11.5 Hz, 26-H), 6.13 (dd, *J* = 11.5, 1.5 Hz, 27-H), 6.31 (dd, *J* = 17.8, 1.5 Hz, 27-H), 3.22 (s, 28-H_3), 3.73 (q, *J* = 7.6 Hz, 29-H_2), 1.70 (t, *J* = 7.6 Hz, 30-H_3), 3.92 (s, 31-H_3), 5.33 (br d, *J* = 18.2 Hz, 33-H), 5.45 (d, *J* = 18.2 Hz, 33-H), 3.67 (s, 34-OMe), benzotriazole moiety 8.00 (br d, *J* = 8.3 Hz, 4'-H), 7.71 (ddd, *J* = 8.3, 6.8, 1.0 Hz, 5'-H), 7.54 (ddd, *J* = 8.3, 6.8, 1.0 Hz, 6'-H), 8.20 (br d, *J* = 8.3 Hz, 7'-H); ^{13}C NMR ($CDCl_3$) δ 167.1 (s, C-1), 52.7 (d, C-2), 49.6 (d, C-3),171.8 (s, C-4), 93.3 (d, C-5), 141.2 (s, C-6), 131.8 (s, C-7), 135.6 (s, C-8), 137.2 (s, C-9), 98.4 (d, C-10), 156.9 (s, C-11), 136.0 (s, C-12), 145.9 (s, C-13), 149.0 (s, C-14), 103.6 (d, C-15), 128.5* (s, C-16), 137.8* (s, C-17), 114.6 (s, C-18), 136.7 (s, C-19), 102.3 (s, C-20), 29.6 (t, C-21), 31.2 (t, C-22), 173.5 (s, C-23), 51.7 (q, 23-OMe), 22.6 (q, C-24), 12.0 (q, C-25), 129.0 (d, C-26), 122.3 (t, C-27), 11.2 (q, C-28), 19.6 (t, C-29), 17.6 (q, C-30), 13.5 (q, C-31), 164.6 (s, C-32), 38.6 (t, C-33), 173.1 (s, C-34), 52.2 (q, 34-OMe), benzotriazole moiety 129.3 (s, C-3'a), 109.3 (d, C-4'), 128.8 (d, C-5'), 124.9 (d, C-6'), 120.5 (d, C-7'), 143.7 (s, C-26)].

32-L-Aspartyl chlorin e6 tetramethyl ester (14): To a solution of **13** (112 mg, 0.151 mmol) in DMF (3 mL) were added L-aspartic acid dimethyl ester hydrochloride (105 mg, 0.531 mmol) and 4-methylmorpholine (58 μL, 0.528 mmol), and the mixture was stirred at 50 °C for 11 h. After cooling, the reaction mixture was diluted with ethyl acetate (20 mL), and washed with 5% aqueous citric acid and brine. After removal of the solvent, the residue was purified by silica gel column chromatography (12 g, hexane - ethyl acetate = 1:1) to give 88.0 mg (75.9%) of **14** as a powder [FAB-MS; *m/z* 768 $(M+H)^+$, Rf 0.31 (TLC 1, hexane - ethyl acetate = 1:1); ^{1}H NMR ($CDCl_3$) δ 4.39 (br dd, *J* = 9.8, 2.2 Hz, 2-H), 4.46 (q, *J* = 7.2 Hz, 3-H), 8.79 (s, 5-H), -1.56 (br, 6-NH), 9.63 (s, 10-H), 9.71 (s, 15-H), -1.74 (br, 16-NH), 1.83 and 2.26 (m, 21-H_2), 2.16 and 2.55 (m, 22-H_2), 3.61 (s, 23-OMe), 1.69 (d, *J* = 7.2 Hz, 24-H_3), 3.49 (s, 25-H_3), 8.09 (dd, *J* = 17.8, 11.5 Hz, 26-H), 6.15 (dd, *J* = 11.5, 1.5 Hz, 27-H), 6.36 (dd, *J* = 17.8, 1.5 Hz, 27-H), 3.32 (s, 28-H_3), 3.81 (q, *J* = 7.7 Hz, 29-H_2), 1.72 (t, *J* = 7.7 Hz, 30-H_3), 3.61 (s, 31-H_3), 7.49 (d, *J* = 8.1 Hz, 32-NH), 5.23 (br d, *J* = 18.8 Hz, 33-H), 5.57 (d, *J* = 18.8 Hz, 33-H), 3.65 (s, 34-OMe), 3.96* (s, 35-OMe), 5.48 (ddd, *J* = 8.1, 4.6, 4.6 Hz, 36-H), 3.33 (dd, *J* = 17.6, 4.6 Hz, 37-H), 3.39 (dd, *J* = 17.6, 4.6 Hz, 37-H), 3.76* (s, 38-OMe); ^{13}C NMR ($CDCl_3$) δ 166.7 (s, C-1), 53.0 (d, C-2), 49.1 (d, C-3),168.9 (s, C-4), 93.7 (d, C-5), 138.9 (s, C-6), 130.2 (s, C-7), 134.5 (s, C-8), 134.9 (s, C-9), 98.8 (d, C-10), 154.3 (s, C-11), 136.1 (s, C-12), 144.8 (s, C-13), 149.0 (s, C-14), 101.5 (d, C-15), 129.8* (s, C-16), 135.2* (s, C-17), 127.1 (s, C-18), 134.9 (s, C-19), 102.3 (s, C-20), 29.7 (t, C-21), 31.1 (t, C-22), 173.5 (s, C-23), 51.6 (q, 23-OMe), 23.1 (q, C-24), 12.2 (q, C-25), 129.5 (d, C-26), 121.7 (t, C-27), 11.4 (q, C-28), 19.7 (t, C-29), 17.7 (q, C-30), 11.9 (q, C-31), 169.1 (s, C-32), 38.1 (t, C-33), 173.4 (s, C-34), 52.1 (q, 34-OMe), 171.1** (s, C-35) , 53.0*** (q, 35-OMe), 49.3 (d, C-36), 36.0 (t, C-37), 171.8** (s, C-38), 52.2*** (q, 38-OMe)].

32-L-Aspartyl chlorin e6 (5): To a solution of **14** (88.0 mg, 0.115 mmol) in 25% aqueous DMF (4 mL) was added 1 M NaOH (1.4 mL) and the mixture was stirred at rt for 12 h. The reaction mixture was diluted with water (20 mL) and washed with dichloromethane (20 mL). The aqueous layer was adjusted pH 2 with aqueous citric acid. After collecting by centrifugation, the obtained black powder was washed with

water and dried at 40℃ to give a crude powder (64.0 mg). The powder was suspended in a small amount of water, to which was added 1 M NaOH (0.36 mL), and the solution was purified by an ODS column chromatography (50 mL) developed with 40% aqueous methanol to afford 62.9 mg (68.6%) of 32-L-aspartyl chlorin e6 (**5**) tetrasodium salt as a powder [TSP-MS; *m/z* 712 $(M+H)^+$ for free acid, Rf 0.23 (TLC 1, chloroform - n-butanol - pyridine - water = 4:4:7:2)].

ACKNOWLEDGMENTS

We thank Mr. Isao Ishikura, Nippon Petrochemicals Co., Ltd., for helpful advice and discussion through this work. We are also grateful to Professor Haruo Seto, Tokyo University, for his kind advice.

REFERENCES AND NOTES

1. J. C. Bommer and B. F. Burnham, CAS No. 105:133666 (EP 168831), CAS No. 105:226204 (EP 168832). Mono-L-aspartyl chlorin e6 (*N*-aspartyl chlorin e6) used in references 2-23 was synthesized essentially by the method of Bommer and Burnham, and is commonly abbreviated to NPe6 or MACE. Mono-L-aspartyl chlorin e6 is chemically pure compound and has been provided from Porphyrin Products, Inc., Beckloff Associates, Inc., (USA), Nippon Petrochemical Co. and Meiji Seika Kaisha, Ltd. (Japan).
2. J. S. Nelson, W. G. Roberts, and M. W. Berns, *Cancer Res.*, 1987, **47**, 4681.
3. K. Aizawa, T. Okunaka, T. Ohtani, H. Kawabe, Y. Yasunaka, S. O'Hata, N. Ohtomo, K. Nishimiya, C. Konaka, H. Kato, Y. Hayata, and T. Saito, *Photochem. Photobiol.*, 1987, **46**, 789.
4. W. G. Roberts, F.-Y. Shiau, J. S. Nelson, K. M. Smith, and M. W. Berns, *J. Natl. Cancer Inst.* 1988, **80**, 330.
5. D. Kessel, *Photochem. Photobiol.*, 1989, **49**, 447.
6. C. J. Gomer and A. Ferrario, *Cancer Res.*, 1990, **50**, 3985.
7. A. Ferrario, D. Kessel, and C. J. Gomer, *Cancer Res.*, 1992, **52**, 2890.
8. J. D. Spikes and J. C. Bommer, *J. Photochem. Photobiol.*, 1993, **58**, 346.
9. T. Katsumi, K. Aizawa, T. Okunaka, Y. Kuroiwa, Y. Ii, K. Saito, C. Konaka, and H. Kato, *Jpn. J. Cancer Res.*, 1994, **85**, 1165.
10. J. Hayashi, T. Saito, H. Sato, Y. Kuroiwa, and K. Aizawa, *Cardiovasc. Res.*, 1995, **30**, 775.
11. W. G. Roberts and M. W. Berns, *Lasers Surg. Med.*, 1989, **9**, 90.
12. W. G. Roberts, L.-H. L. Liaw, and M. W. Berns, *Lasers Surg. Med.*, 1989, **9**, 102.
13. D. Kessel, K. L. Whitcomb, and V. Schulz, *Photochem. Photobiol.*, 1992, **56**, 51.
14. J. D. Spikes and J. C. Bommer, *J. Photochem. Photobiol. B. Biol.*, 1993, **17**, 135.
15. J. Hayashi, Y. Kuroiwa, H. Sato, T. Saito, and K. Aizawa, *Cardiovasc. Res.*, 1993, **27**, 1943.
16. K. S. McMahon, T. J. Wieman, P. H. Moore, and V. H. Finger, *Cancer Res.*, 1994, **54**, 5374.
17. D. Kessel, *J. Photochem. Photobiol. B. Biol.*, 1995, **28**, 13.
18. D. Kessel, *Photochem. Photobiol.*, 1995, **61**, 646.
19. C. J. Gomer, *J. Clin. Laser Med. Surg.*, 1996, **14**, 315.
20. T. A. Katsumi, K. Aizawa, Y. Kuroiwa, K. Saito, Y. Kurata, Y. Ii, T. Okunaka, C. Konaka, and H. Kato, *Photochem. Photobiol.*, 1996, **64**, 671.
21. J. Hayashi, T. Saito, and K. Aizawa, *Lasers Surg. Med.*, 1997, **21**, 287.
22. D. Kessel, *J. Photochem. Photobiol. B. Biol.*, 1997, **39**, 81.
23. L.-M. Wong Kee Song, K. K. Wang, and A. R. Zinsmeister, *Cancer*, 1998, **82**, 421.
24. H. Fischer and M. Strell, *Ann.*, 1940, **543**, 157.

25. R. B. Woodward, W. A. Ayer, J. M. Beaton, F. Bickelhaupt, R. Bonnett, P. Buchschacher, G. L. Closs, H. Dutler, J. Hannah, F. P. Hauck, S. Itô, A. Langemann, E. Le Goff, W. Leimgruber, W. Lwowski, J. Sauer, Z. Valenta, and H. Volz, *J. Am. Chem. Soc.*, 1960, **82**, 3800.
26. T. Ando, K. Irie, K. Koshimizu, T. Takemura, H. Nishino, A. Iwashima, S. Nakajima, and I. Sakata, *Tetrahedron*, 1990, **46**, 5921.
27. H. H. Inhoffen, G. Klotmann, and G. Jeckel, *Liebigs Ann. Chem.*, 1947, **557**, 186.
28. J. W. Purdie and A. S. Holt, *Can. J. Chem.*,1965, **43**, 3347.
29. J. L. Archibald, D. M. Walker, K. B. Shaw, A. Markovac, and S. F. MacDonald, *Can. J. Chem.*, 1966, **44**, 345.
30. K. M. Smith, G. M. F. Bisset, and M. Bushell, *Bioorg. Chem.*, 1980, **9**, 1.
31. G. R. Seely, A. S. Holt, and W. Lwowski, 'The Chlorophylls', ed. by L. P. Vernon and G. R. Seely, Academic Press, Inc., New York, 1966, pp. 67-146.
32. J. Gassmann, I. Strell, F. Brandl, M. Sturm, and W. Hoppe, *Tetrahedron Lett.*, 1971, 4609.
33. M. S. Fischer, D. H. Templeton, A. Zalkin, and M. Calvin, *J. Am. Chem. Soc.*, 1972, **94**, 3613.
34. M. O. Senge, K. R.-Senge, and K. M. Smith, *Acta Cryst.*, 1992, **C48**, 1810.
35. K. M. Barkigia, D. S. Gottfried, S. G. Boxer, and J. Fajar, *J. Am. Chem. Soc.*, 1989, **111**, 6444.
36. K. M. Barkigia, J. Fajer, K. M. Smith, and G. J. B. Williams, *J. Am. Chem. Soc.*, 1981, **103**, 5890.
37. H.-C. Chow, R. Serlin, and C. E. Strouse, *J. Am. Chem. Soc.*, 1975, **97**, 7230.
38. R. Serlin, H.-C. Chow, and C. E. Strouse, *J. Am. Chem. Soc.*, 1975, **97**, 7237.
39. K. M. Smith and D. A. Goff, *J. Am. Chem. Soc.*, 1983, **105**, 1674.
40. K. M. Smith, D. A. Goff, J. Fajer, and K. M. Barkigia, *J. Am. Chem. Soc.*, 1982, **104**, 3747.
41. R. J. Abraham, G. E. Hawkes, and K. M. Smith, *Chem. Soc. Perkin II*, 1974, 627.
42. K. M. Smith and J. F. Unsworth, *Tetrahedron*, 1975, **31**, 367.
43. D. Brühwiler and G. Wagner, *J. Magn. Reson.*, 1986, **69**, 546.
44. A. Bax and M. F. Summers, *J. Am. Chem. Soc.*, 1986, **108**, 2093.
45. L. M. Jackman, F. Sondheimer, Y. Amiel, D. A. Ben-Efraim, Y. Gaoni, R. Wolovsky, and A. A. Bothner, *J. Am. Chem. Soc.*, 1962, **84**, 4307.
46. L. Lerner and A. Bax, *J. Magn. Reson.*, 1986, **69**, 375.
47. G. L. Closs, J. J. Katz, F. C. Pennington, M. R. Thomas, and H. H. Strain, *J. Am. Chem. Soc.*, 1963, **85**, 3809.

Received, 9th July, 1998