HETEROCYCLES, Vol. 103, No. 2, 2021, pp. 1108 - 1115. © 2021 The Japan Institute of Heterocyclic Chemistry
Received, 28th December, 2020, Accepted, 2nd February, 2021, Published online, 15th March, 2021
DOI: 10.3987/COM-20-S(K)73

LARGE-SCALE SYNTHESIS OF THIO-GLUCOSE-CONJUGATED CHLORIN E6 FOR PHOTODYNAMIC THERAPY

Akihiro Nomoto,[a]* Hiroaki Yamaguchi,[a] Masato Masuda,[a] Keisuke Hyakumura,[a] Shintaro Kodama,[a] Tomohiro Osaki,[b] Yoshiharu Okamoto,[b] Mamoru Tanaka,[c] Hiromi Kataoka,[c] Atsushi Narumi,[d] Tomokazu Yoshimura,[e] Shigenobu Yano,[e] and Akiya Ogawa[a]

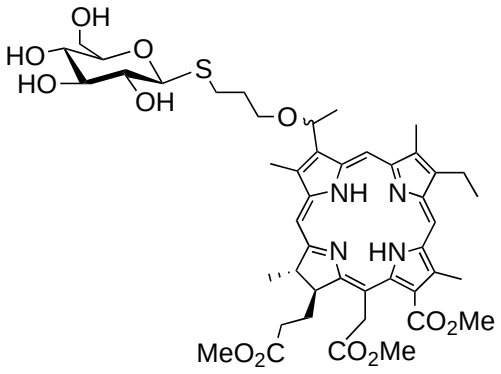
[a]Department of Applied Chemistry, Graduate School of Engineering, Osaka Prefecture University, 1-2, Gakuen-cho, Sakai, Osaka 599-8570, Japan, E-mail: nomoto@chem.osakafu-u.ac.jp.
[b]Joint Department of Veterinary Clinical Medicine, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan.
[c]Department of Gastroenterology and Metabolism, Nagoya City University Graduate School of Medical Sciences, 1 Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan.
[d]Graduate School of Organic Materials Science, Yamagata University, 4-3-16 Jonan, Yonezawa, Yamagata 992-8510, Japan.
[e]KYOUSEI Science Center for Life and Nature, Nara Women's University, Kitauoyahigashi-machi, Nara 630-8506, Japan.

This paper is dedicated to Professor Yasuyuki Kita on the occasion of his 77th birthday.

Abstract – Chlorin e6 is a heterocycle exhibiting spectral absorption in the 600–700 nm wavelength range used for photodynamic therapy (PDT). Herein, a sugar-conjugated chlorin e6 derivative was synthesized on a large scale. An alkyl spacer was fabricated by controlling the alkoxylation conditions between the thio-sugar and chlorin e6 and thio-glucose-conjugated chlorin e6 was successfully synthesized.

Heterocycles such as porphyrins and chlorins show great potential to be developed as new drugs. Therefore, it is important to develop new synthesis methods and pathways for drugs to fight novel

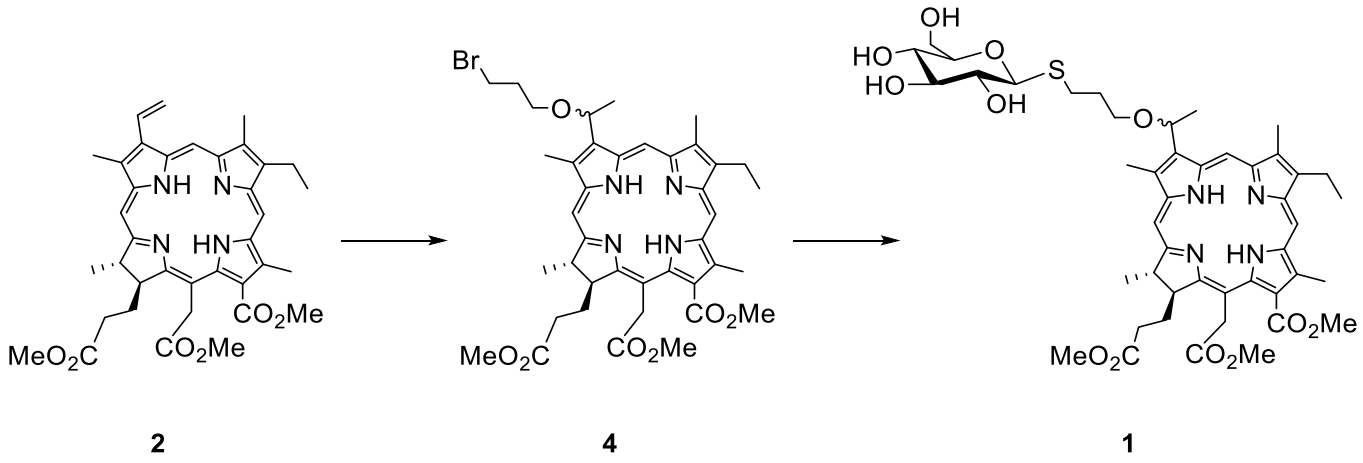
diseases of the future. Chlorin derivatives have remarkable photoproperties. They show absorption in the visible light region (approximately 650 nm) and are used for the photodynamic therapy (PDT). PDT has attracted much attention of late because it has shown promise as a noninvasive treatment for cancer.[1] PDT involves the administration of a photosensitizer to the patient. The photosensitizer is then activated by photoirradiation to convert triplet oxygen ($^3O_2$) into reactive singlet oxygen species ($^1O_2$), which damages or kills cancer cells. Although this activation occurs selectively at the photoirradiated tumor site,[2] the use of photosensitizers has several disadvantages as well, such as increase in the photosensitivity of the skin. In some reported photosensitizers based on chlorin, carboxyl groups were used mainly for modifications since condensation reagents can be applicable to incorporation. In contrast, the alkenyl moiety in chlorin e6 undergoes only a minor transformation. Therefore, in this work, we have investigated the synthesis of effective photosensitizers and the effects of glucose-conjugated chlorin e6 as a photosensitizer for PDT (Scheme 1).[3] General pharmacokinetic study, safety test, and animal experiments require large amount of the photosensitizer. Thus in this work, a large-scale synthesis of thio-β-D-glucose-conjugated chlorin e6 trimethyl ester (**1**) was investigated in detail.



1

Scheme 1. Schematic representation of thio-β-D-glucose-conjugated chlorin e6 trimethyl ester (**1**)

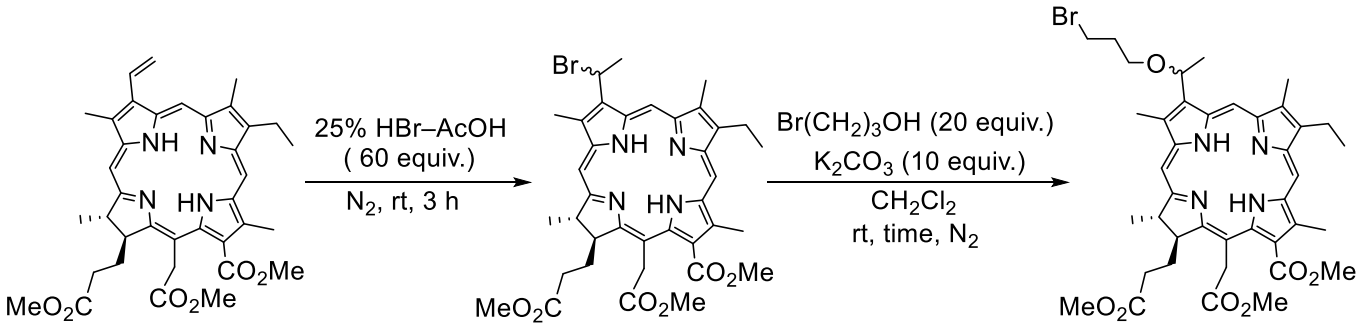
We expected the incorporation of a sugar moiety into chlorin e6 might improve its affinity for cancer cells. An alkyl spacer was also needed for this conjugation because steric hindrance prevented the sugar moiety from connecting directly to the heterocycle. We initially chose to synthesize **1**, which has a C3 spacer. We also synthesized a derivative of **1** with a C4 spacer on a small scale, but a preliminary in vivo study indicated that the PDT effect of **1** is slightly better than that of the C4 derivative. (The reason for this is not clear and currently under investigation.) As a result of its higher performance, we decided to synthesize **1** on a large scale for use in future studies. Commercially available chlorin e6 trimethyl ester (**2**) was first subjected to haloalkoxylation and subsequently to thioglycosylation to obtain the desired product (**1**) (Scheme 2).



Scheme 2. Synthetic pathway of thio-β-D-glucose-conjugated chlorin e6 trimethyl ester (**1**)

One-pot alkoxylation of **2** was optimized, which involves two steps: bromination of alkene, followed by alkoxylation with alcohol (Scheme 3). The starting **2** was dissolved in 25% HBr-AcOH solution, and this reaction mixture was stirred at ambient temperature under N_2 atmosphere.[4] The reaction progress was monitored by TLC because **3** is not stable. After 3 h, the solvent, acetic acid (AcOH), was removed in vacuo to obtain a black viscous slurry. This removal of AcOH is not difficult for small-scale synthesis; however, when the synthesis was carried out on a large scale, a small amount of AcOH remains in the reaction mixture and an acetyl group-substituted by-product is formed instead of an alkyl group-substituted product (**4**) in the following step. Although the desired product (**3**) can also be obtained by using HBr gas in the CH_2Cl_2 solvent without AcOH solvent, we synthesized **3** using 25% HBr-AcOH because of its commercial availability.

To remove AcOH as much as possible, CH_2Cl_2 was added and evaporated three times to obtain **3** as a black powder. To perform alkoxylation at a small scale, CH_2Cl_2, 3-bomopropan-1-ol, and K_2CO_3 were added at 0 °C and then stirred at ambient temperature under N_2 atmosphere according to the literature.[5] The crude product was purified by flash column chromatography ($CHCl_3$:AcOEt = 20:1). A scale-up of this reaction is summarized in Scheme 3. The reaction yield of **4** decreased with the increase of the amounts of **2**. The reaction is not affected by the amount of the solvent and additive (entries 1-4). However, the yield of **4** decreased on prolonging the reaction time (entries 5 and 8). We assumed that K_2CO_3 mainly formed AcO^- by remained AcOH and afforded only the acetylated by-product (entry 9); therefore, we gradually added K_2CO_3 and noted an improved reaction yield (entry 7). These reaction conditions were also applicable to the large-scale synthesis (entry 10, 10.9 g) and the desired product (**4**) was obtained in 68% (9.0 g) yield.



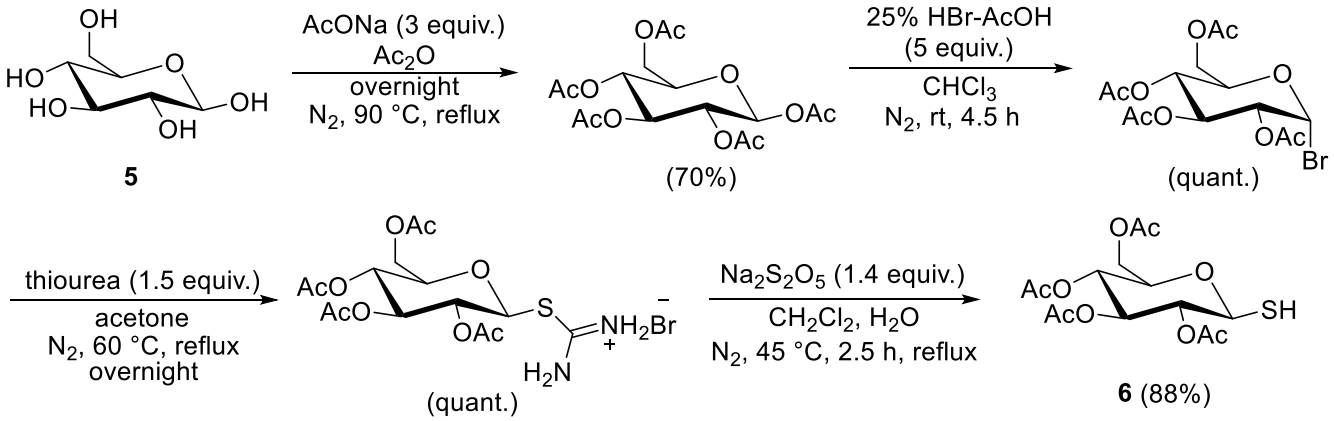
2 **3** **4**

entry	**2** (μmol)	KCl (equiv.)	time (h)	CH_2Cl_2 (mL)	yield (%)
1	32	–	5	1	46
2	94	–	5	4	32
3	94	18.5	5	4	38
4	391	–	5	14	38
5	800	–	66.5	30	28
6	1500	–	3	40	49
7[a]	5000	–	4	125	68
8[b]	7500	–	4.3	190	N.D.
9	8000	–	4.3	200	N.D.
10[a]	17000	–	4	420	68

[a]$Br(CH_2)_3OH$ was added before adding K_2CO_3.

[b]The reaction time of first step was 7.5 h.

Scheme 3. Optimization reaction condition for alkoxylation

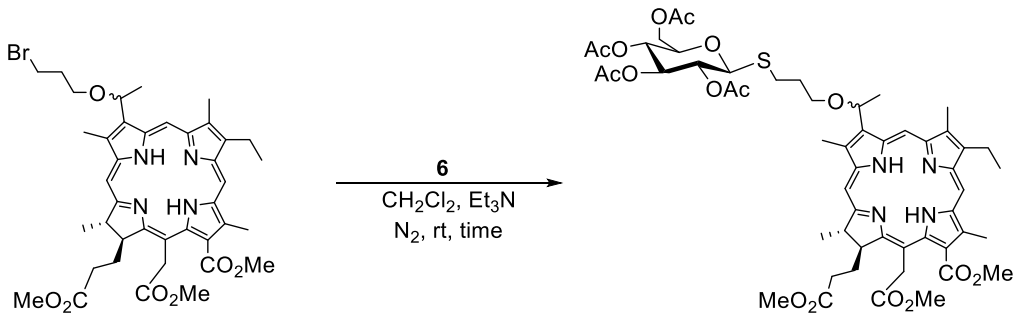


Scheme 4. Schematic representation of 1-thio-β-D-glucose tetraacetate synthesis

We next examined incorporation of a glucose moiety into **4**. The acetyl-protected thio-β-D-glucose (**6**) was obtained from β-D-glucose (**5**) according to a previously reported 10 g-scale synthesis of 1-thio-β-D-glucose tetraacetate.[6] Each reaction proceeded smoothly, and the desired product (**6**) was obtained as a white solid (~20 g) (Scheme 4). Compound **6** was then reacted with the prepared bromoalkoxylated chlorin **4**; the reaction conditions are summarized in Scheme 5. Compound **7** was isolated by column chromatography, although the reaction mixture still contained a small amount of starting material **6** as determined by NMR and mass spectroscopic studies. When 3.3 equiv. of **6** was used, the yield of **7** were obtained in 40% and 7%, respectively (entries 1 and 2). Compared to this result, the use of 4.5 equiv. of **6** afforded **7** in moderate yields (entries 3–5, and 7). Prolonging the reaction time was not effective in increasing the yield (entry 4), although the yield improved when 12 equiv. of Et_3N was used (entry 5–7). Under these optimized conditions, **7** was obtained in 71% yield when 9.0 g (11.5 mmol) of **4** was used.

Finally, deacetylation was carried out under ambient conditions;[7] the results are shown in Scheme 6. The reaction progress was investigated by TLC; moderate yields were obtained in each case, and the UV-vis spectrum of **1** showed remarkable absorption at 655 nm (Figure 1). Through the developed synthesis method of **1**, we could obtain a large amount of products successfully.

In summary, the desired final product was obtained in 5.7 g (6.4 mmol) using 10.9 g (17 mmol) of chlorin e6 (**2**). The method developed here for large-scale synthesis of **1** has made it possible to carry out safety tests in vivo. We are currently investigating in vivo studies with mice based on common methods, and we would like to point out that we did not observe any noticeable toxicity in the prior safety tests. Some heterocycles also have rich potentials as new medical drugs. Thus, development of synthesis methods of heterocycles will become more and more important for new diseases in the future.



4 **7**

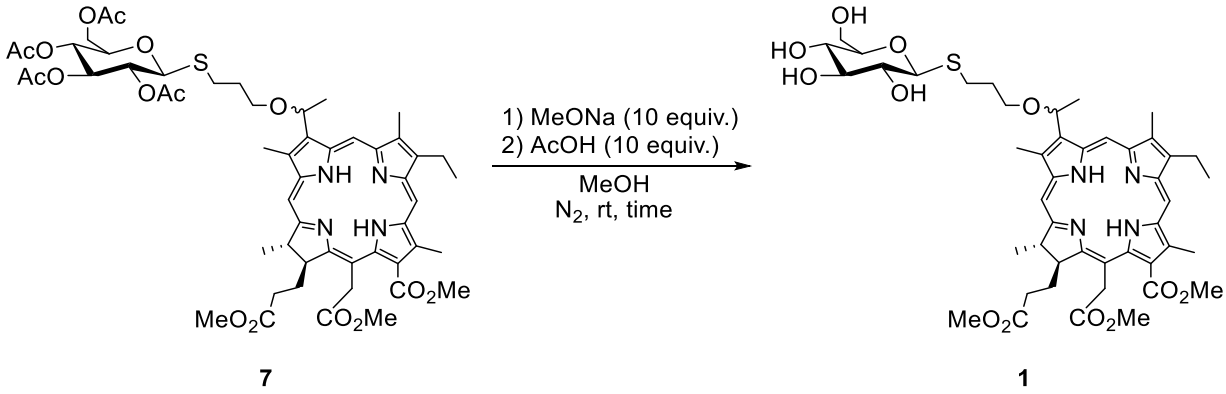
entry	**4** (mmol)	**6** (equiv.)	time (h)	yield (%)
1[a]	0.132	3.3	3.5	40
2[a]	0.422	3.3	3.5	7
3[a]	3.00	4.5	5.0	56

4[a]	3.00	4.5	22	46
5[b]	1.30	4.5	3.5	82
6[b]	4.61	3.5	4.0	68
7[b]	11.5	4.5	3.5	71

[a]Et_3N (6 equiv.) was added.

[b]Et_3N (12 equiv.) was added.

Scheme 5. Optimization for incorporation of acetylated thio-β-D-glucose



7 **1**

entry	**7** (μmol)	MeOH (mL)	time (h)	yield (%)
1	53	5	2.0	45
2	500	40	0.5	60
3	4620	380	0.7	70
4	4950	400	1.0	63

Scheme 6. Optimization of de-acetylation reaction affording **1**

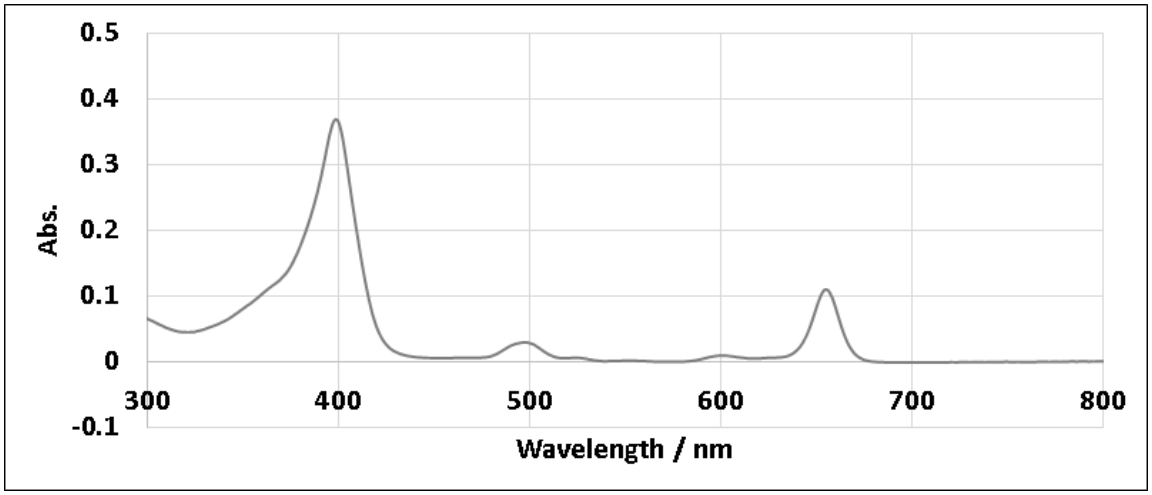


Figure 1. UV-vis. spectrum of **1** (3×10^{-6} M in DMSO)

EXPERIMENTAL

GENERAL

^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-AL-300 or JEOL JNM-AL-400 spectrometer using $CDCl_3$ as the solvent and tetramethylsilane (TMS) as an internal standard. MALDI-TOF-MS mass spectra were measured on JEOL JMS-T100LC and Bruker Autoflex II. All materials were obtained from commercial suppliers (WAKO Co., Ltd. and Funakoshi Co., Ltd.) and used without further purification.

3^1-(3-Bromopropoxy)chlorin e6-trimethyl ester (**4**)

2 (5.00 mmol, 1.0 equiv) was dissolved in 25% HBr-AcOH solution (300 mmol, 60 equiv). The mixture was then stirred for 3 h at ambient temperature under N_2 atmosphere. After the reaction, the solvent was removed in vacuo. The product was dissolved in 125 mL of CH_2Cl_2, and then, 3-bromopropan-1-ol (100 mmol, 20 equiv.) and K_2CO_3 (50.0 mmol, 10 equiv.) were added at 0 °C. The resulting mixture was stirred for 4 h at ambient temperature under N_2 atmosphere. After removing K_2CO_3 by suction filtration, the organic layer was washed with distilled water and saturated aqueous NaCl solution and dried over anhydrous Na_2SO_4. The crude product was purified by flash column chromatography on silica gel using 5% AcOEt in $CHCl_3$.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.79 (d, *J* = 4.4 Hz, 1H), 9.70 (s, 1H), 8.72 (s, 1H), 5.91 (dd, *J* = 6.0 Hz and 19.2 Hz, 1H), 5.30 (dd, *J* = 27.2 Hz and 64.8 Hz, 2H), 4.39–4.46 (m, 2H), 4.25 (s, 3H), 3.73–3.84 (m, 9H), 3.60 (s, 3H), 3.58 (s, 3H), 3.41 (d, *J* = 4.0 Hz, 3H), 3.30 (s, 3H), 2.46–2.60 (m, 1H), 2.08–2.23 (m, 7H), 1.68–1.75 (m, 7H), 0.84–1.25 (m, 1H), -1.50–1.33 (m, 2H)

^{13}C NMR (400 MHz, $CDCl_3$): δ = 173.7, 173.2, 169.8, 169.7, 166.8, 154.8, 149.0, 145.2, 139.3, 138.3, 136.6, 136.1, 135.3, 135.0, 134.7, 134.5, 131.2, 131.1, 129.4, 123.5, 102.3, 99.2, 93.4, 76.9, 67.1, 53.2, 53.0, 52.3, 51.8, 49.4, 38.7, 33.5, 31.2, 30.9, 29.7, 24.9, 24.8, 23.0, 19.8, 17.9, 12.5, 11.5, 11.3. MALDI-TOF mass ($C_{40}H_{49}BrN_4O_7$) *m/z* 776.

3^1-(3-(1-Thio-β-D-glucopyranosyl)propoxy)chlorin e6-trimethyl ester (**1**)

Et_3N (53 mmol, 12 equiv) was added to a solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (16.1 mmol, 3.5 equiv.) in 30 mL of CH_2Cl_2. A solution of **4** (4.61 mmol, 1.0 equiv.) in 30 mL of CH_2Cl_2 was added dropwise at 0 °C and the mixture was stirred for 3.5 h at ambient temperature under N_2 atmosphere. The organic layer was washed with distilled water and saturated aqueous NaCl solution and dried over anhydrous Na_2SO_4. The crude product was purified by flash column chromatography on silica gel using 9% AcOEt in $CHCl_3$. MALDI-TOF ($C_{54}H_{68}N_4O_{16}S$, (**7**)) *m/z* 1060. This product (4.62 mmol) was dissolved in 380 mL of MeOH, and MeONa (47 mmol, 10 equiv.) was added.[8] The reaction mixture was stirred for 0.5 h at ambient temperature under N_2 atmosphere. The resulting mixture was quenched

with AcOH (47 mmol, 10 equiv.). The solvent was removed in vacuo, and the crude product was purified by flash column chromatography on silica gel using 9% MeOH in $CHCl_3$.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.76 (d, *J* = 13.2 Hz, 1H), 9.63 (d, *J* = 5.2 Hz, 1H), 8.68 (d, *J* = 2.4 Hz, 1H), 5.78–5.87 (m, 1H), 5.27 (q, *J* = 27.2 Hz, 2H), 4.34–4.42 (m, 2H), 4.22 (s, 3H), 3.76 (d, *J* = 5.6 Hz, 3H), 3.75–3.66 (m, 3H), 3.62–3.58 (m, 6H), 3.57–3.59 (m, 4H), 3.56–3.53 (m, 4H), 3.51–3.41 (m, 2H), 3.34 (s, 3H), 3.23 (s, 3H), 3.14 (t, *J* = 8.8 Hz, 1H), 3.03–3.04 (m, 1H), 2.74–2.89 (m, 2H), 2.58–2.68 (m, 1H), 2.51–2.58 (m, 1H), 2.30–2.41 (m, 1H), 2.04–2.12 (m, 3H), 2.00–1.60 (m, 9H), -1.60 (s, 2H). mp>250 °C (decomp.). Anal. Calcd for (**1**·CH_2Cl_2·MeOH): C. 57.08%; H. 6.59%; N. 5.55%. Found: C. 57.23%; H. 6.26%; N. 5.87%. HR-ESI mass ($C_{46}H_{60}N_4O_{12}S$): calcd. 892.3928; found. 892.3895.

ACKNOWLEDGEMENTS

This research was supported by JSPS KAKENHI (B, 19H02791; C, 20K08391, 18K05161; Young Scientists, 20K16965) from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and by the Kyoto-Advanced Nanotechnology Network. We also thank Prof. Shigeyuki Yagi and Associate Prof. Takeshi Maeda (mass spectroscopy).

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