Supplementary Information

Contents

1. Experimental Section

1.1 Materials

Maltotriose (Mal_3, 97.0 %) was kindly supplied from Hayashibara Biochemical Laboratories, Inc., Japan, and used as received. 5,10,15,20-Tetrakis(perfluorophenyl)porphyrin (TFPP) and diethylamine (Et_2NH) were purchased from Tokyo Chemical Industry Co., Japan. 5,10,15,20-Tetrakis(pentafluorophenyl)-2,3-[methano(*N*-methyl)iminomethano]chlorin[1] (TFPC) and 1-thioacetyl-2,3,6-tri-*O*-acetyl-4-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-*α*-*O*-glucopyranosyl)-*α*-D-glucopyranosyl)-*β*-D-glucopyranose[2, 3] ($AcMal_3$-SAc) were prepared according to the literature procedures. Dry *N*,*N*-dimethylformamide (DMF), dry tetrahydrofuran (THF), and dry methanol (MeOH) were prepared by the distillations of the commercially available solvents using drying agents of CaH_2, Na, and Mg, respectively. Sodium methoxide (NaOMe, 2.0 wt% in dry THF) was prepared from NaOMe (ca. 5 mol/L in methanol, Wako Pure Chemical Industries, Japan) and dry THF. Deionized water (electrical conductivity = 18.3 MΩ cm) was prepared by Advantec Aquarius GSH-5000 and Millipore Milli-Q Jr. Silica gel 60 F_{254} (TLC plates), silica gel 60 RP-18 F_{254}S (TLC plates), silica gel 60N (spherical, neutral pH, 63 ~ 210 μm), and silica gel 120 RP-18 (spherical, 40 ~ 50 μm) were purchased from Kanto Chemical Co., Japan. 9,10-Anthracenediyl-bis(methyl)dimaloinic acid (ABDA, >90 %) was purchased form Aldrich. All other chemicals were obtained from commercial sources and used as received unless otherwise stated.

1.2 Measurements

The ^1H, ^{13}C, and ^{19}F NMR spectra were recorded using a JEOL JNM-ECX400 instrument. Infrared (IR) spectra were recorded using a HORIBA FT-720 spectrometer. UV-vis spectra were recorded on a JASCO V-500 spectrophotometer. The electrospray ionization time-of-flight mass spectrometry (ESI-TOF MS) measurement was performed by a JEOL JMS-T100LC AccuTOF LC.

1.3 LC-MS Measurements

The liquid chromatography (LC) was performed using a Phenomenex Kinetex 2.6 μm column (XB-C18) and an ultraviolet (UV) detector (404 nm) in 10 mM ammonium acetate aq./MeOH (60/40 ~ 0/100). The mass spectrometry (MS) measurement was performed by Waters Synapt G2 (Ionization method, ESI+; Scan range, m/z = 500 ~ 3000).

1.4 Detection of Singlet Oxygen

The detection of $^1O_2$ generation using ABDA was conducted in accordance with methods previously described in the literature.[4] For the ABDA bleaching method, all of the samples were prepared using a deuterium oxide solution. The concentrations of the porphyrin derivatives and ABDA in the mixed solutions were [porphyrin derivatives] = 15 μM and [ABDA] = 25 μM. Oxygen was bubbled through all of the samples for 15 min prior to their photoirradiation to generate the necessary aerobic conditions. The photoirradiation was performed using a xenon lamp (SX-UID500X, 500 W; Ushio Inc., Tokyo, Japan) equipped with a long-pass filter with a cut-off at 600 nm. The light was cooled by passing it through a water filter. The power of the light was 15 mW cm^{-2} (over 600 nm) at the sample level.

1.5 Near-IR Emission Measurements

Singlet oxygen generation was detected by its phosphorescence emission at 1270 nm. An O_2-saturated D_2O solution containing a photosensitizer in a quartz cell (optical path length 10 mm) was excited by a laser at 532 nm (5 mW) using a HORIBA LabRAM HR Evolution emission spectrophotometer.

1.6 Photodynamic Activity Experiments

The PDT test was conducted according to the literature.[5] HeLa cells were maintained in a minimum essential medium (MEM; Thermo Fisher Science, Massachusetts, USA) supplemented with 10% fetal

calf serum at 37 °C in 5% CO_2. For the experiments conducted to determine the photodynamic activities of Mal_3-chlorin, the cells were seeded in 48-well culture plates at a density of 8.55×10^4 cells per well. After growing overnight, the cells were incubated with Mal_3-chlorin for 24 h in the absence of light. The cells were washed with PBS and exposed to light for 30 min at 25 °C. Light irradiation was performed using a xenon lamp (MAX-301, 300 W; Asahi Spectra Co., Ltd., Osaka, Japan) equipped with a VIS mirror module (385−740 nm) and a long-pass filter with a cut-off of 610 nm. The power of the light at the cellular level was 9 mW cm^{-2} (610–740 nm). To measure the viability of cells as a percentage ratio relative to the cells that were not treated, a WST-8 assay was conducted 24 h after the photoirradiation process using a Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions.

1.7 Fluorescence Microscopy of HeLa Cells

Following a 24-h period of incubation with 4.0 μM of Mal_3-chlorin or Mal_3-porphyrin at 37 °C, the cells were replaced with fresh medium and the introduction of Mal_3-chlorin or Mal_3-porphyrin was monitored by fluorescence microscopy. The cells were observed using an Olympus IX71 epifluorescence microscope equipped with a 200 × objective lens. Fluorescent images were recorded using a Hamamatsu ImagEM EM-CCD camera (C9100-13) under irradiation of an excitation light beam from a mercury lamp through an optical filter (U-MNIBA2, Olympus).

1.8 Identification of Reactive Oxygen

Following the treatment with Mal_3-chlorin (3 μM), the cells were incubated with 50 mM L-histidine (Kishida Chemical Co., Ltd.) or D-mannitol (Wako Pure Chemical Industries Ltd.) for 30 min. After incubation, the cells were exposed to light in the presence of the scavengers and the effect of the presence of the scavengers on the cell viability was determined.

1.9 Synthesis of 5,10,15,20-tetrakis[4-(deca-*O*-acetyl-*β*-D-maltotriosylthio)-2,3,5,6-tetrafluorophenyl]porphyrin (AcMal$_3$-porphyrin)

To the solution of TFPP (0.14 g, 0.14 mmol) and AcMal$_3$-SAc (0.58 g, 0.59 mmol) in dry DMF (64 mL), Et$_2$NH (6.4 mL) was dropwise added. After being stirred for 2 hours at room temperature under a nitrogen atmosphere, the mixture was transferred to a separating funnel, and $CHCl_3$ (100 mL) was added. The organic layer was washed with water (30 mL × 10) and dried over sodium sulfate and then evaporated to dryness. The residue was purified by flash column chromatography (silica gel; $CHCl_3$/EtOAc, 2:3→1:4) to give AcMal$_3$-porphyrin as a brownish-red solid (0.21 g, 32 %). R_f = 0.67 ($CHCl_3$/EtOAc, 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$, Si $(CH_3)_4$ = 0 ppm) : δ (ppm) = 9.04 (8H, s, *β*-pyrrole *H*), 5.44-5.33 (20H, m, *H*-1$^{Mal2\text{-}Mal3}$, *H*-3$^{Mal1\text{-}Mal3}$), 5.18 (4H, d, $J_{1,2}$ = 10.1 Hz, *H*-1^{Mal1}), 5.09-5.01 (8H, m, *H*-2^{Mal}, *H*-4^{Mal3}), 4.86 (4H, dd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.4 Hz,*H*-2^{Mal2}), 4.77 (4H, dd, $J_{1,2}$ = 3.6 Hz, $J_{2,3}$ = 10.0 Hz,*H*-2^{Mal3}), 4.70-4.35 (12H, m, *H*-6$^{Mal1\text{-}Mal3}$), 4.26-3.92 (32H, m, *H*-4$^{Mal1\text{-}Mal2}$, *H*-5$^{Mal1\text{-}Mal3}$, *H*-6'$^{Mal1\text{-}Mal3}$), 2.18-2.00 (120H, s, C*H*$_3$), -2.89 (brs, 2H, N*H*). ^{19}F-NMR (376 MHz, $CDCl_3$): δ (ppm) = -131.53 (8F, s, 3,5-Ph*F*), -135.98 (8F, s, 2,6-Ph*F*). ^{13}C-NMR (100 MHz, $CDCl_3$ = 77 ppm) : δ (ppm) = 170.72, 170.62, 170.50, 170.43, 170.24, 169.97, 169.88, 169.83, 169.55 (-O*C*OCH$_3$), 148.05 (2,6-Ph-*C*), 145.54 (3,5-Ph-*C*), 131.62 (*α*-pyrrole *C*, *β*-pyrrole *C*), 122.30 (4-Ph-*C*), 111.49 (1-Ph-*C*), 104.34 (*meso C*), 95.75, 95.96 (*C*-1$^{Mal2\text{-}Mal3}$), 83.91 (*C*-1^{Mal1}), 73.40, 72.42, 71.82, 71.32, 70.58, 70.14, 69.40, 69.19, 68.61, 67.95 (*C*-2$^{Mal1\text{-}Mal3}$, *C*-3$^{Mal1\text{-}Mal3}$, *C*-4$^{Mal1\text{-}Mal3}$, *C*-5$^{Mal1\text{-}Mal3}$), 62.57, 62.31, 61.44 (*C*-6$^{Mal1\text{-}Mal3}$), 21.08, 21.03, 20.09, 20.86, 20.78, 20.70 (-OCO*C*H$_3$). FT-IR (KBr): ν (cm^{-1}) = 1753, 1471, 1373, 1232, 1041, 974. ESI-TOF MS (*m*/*z*) calcd for $[M + H]^+$,4656.04; found, 4656.40. UV-vis (*c* = 5.00 μM, DMSO, path length = 1 cm, 25 ºC): *λ* / nm (*ε* × 10^{-3} /M^{-1} cm^{-1}) = 415 (278), 507 (19.4), 539 (2.98), 580 (6.43), 634 (0.74).

1.10 Synthesis of 5,10,15,20-tetrakis[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]-2,3-[methano(*N*-methyl)iminomethano]chlorin (AcG-chlorin)

The same procedure as that used for the preparation of $AcMal_3$-chlorin was applied to TFPC (0.15 g, 0.15 mmol) and AcGlc-SAc (0.27 mg, 0.66 mmol) ([TFPC]:[AcGlc-SAc] = 1.0/4.4) to give AcG-chlorin as a green solid (0.21 g, 58 %). ^{1}H-NMR (400 MHz, $CDCl_3$, Si $(CH_3)_4$ = 0 ppm) : δ (ppm) = 8.82 (2H, t, ^3J = 5.5 Hz, 8,17-*β*-pyrrole *H*), 8.56 (2H, s, 12,13-*β*-pyrrole *H*), 8.54-8.47 (2H, m, 5.0 Hz, 7,18-*β*-pyrrole *H*), 5.36-5.32 (4H, m, 3-Glc*H*), 5.34 (2H, m, 2,4-*β*-pyrrole *H*), 5.24-5.08 (12H, m, 1,2,4-Glc*H*), 4.32-4.28 (8H, m, 6-Glc*H*), 3.87 (4H, m, 5-Glc*H*), 2.22 (3H, brs, CH_3), 2.21-2.06 (48H, s, CH_3), -1.77 (2H, brs, N*H*).

1.11 Synthesis of 5,10,15,20-tetrakis[4-(2,3,4,6-tetra-*O*-acetyl-*β*-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl] porphyrin (AcG-porphyrin). A literature procedure was modified. A mixture of TFPP (0.12 g, 0.13 mmol) and Et_2NH (0.20 mL) in dry DMF (47 mL) was stirred at 0 ºC under a nitrogen atmosphere. To the mixture, AcGlc-SAc (0.27 g, 0.67 mmol) in dry DMF (10 mL) was dropwise added ([TFPP]:[AcGlc-SAc] = 1.0/5.2). After being stirred for 2 hours at room temperature under a nitrogen atmosphere, the mixture was transferred to a separating funnel, $CHCl_3$ (100 mL) and water (100 mL) were added, and the organic layer was separated. The aqueous layer was extracted with chloroform (50 mL × 3) and the combined organic layers were washed with water (50 mL) and dried over sodium sulfate and then evaporated to dryness. The residue was purified by flash column chromatography (silica gel; hexane/EtOAc, 2:3) to give AcG-porphyrin as a purple solid (0.23 g, 78 %). R_f = 0.23 (hexane/EtOAc, 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$, Si $(CH3)_4$ = 0 ppm) : δ (ppm) = 9.02 (8H, s, β-pyrrole *H*), 5.38 (4H, t, ^3J = 9.2 Hz, 3-Glc*H*), 5.22-5.28 (8H, m, 2,4-Glc*H*), 5.17 (4H, d, ^3J = 10.1 Hz, 1-Glc*H*), 4.32 (8H, d, ^3J = 3.2 Hz, 6-Glc*H*), 3.90 (4H, dt, ^3J = 9.6 Hz, 3.7 Hz , 5-Glc*H*), 2.23 (12H, s, CH_3), 2.10 (12H, s, CH_3), 2.09 (12H, s, CH_3), 2.08 (12H, s, CH_3).

1.12 Synthesis of 5,10,15,20-tetrakis[4-(*β*-D-maltotriosylthio)-2,3,5,6-tetrafluorophenyl]porphyrin (Mal$_3$-porphyrin)

The same procedure as that used for the preparation of Mal$_3$-chlorin was applied to AcMal$_3$-porphyrin (43 mg, 9.1 μmol) and the formation of the target product was confirmed by TLC (CH_3CN/H_2O = 1/1, R_f = 0.71) to give Mal$_3$-porphyrin as a brownish-red solid (21 mg, 78 %). FT-IR (KBr): ν (cm^{-1}) = 3400, 1468, 1028. UV-vis (*c* = 5.00 μM, H_2O, path length = 1 cm, 25ºC): λ/nm (ε × $10^{-3}/M^{-1}$ cm^{-1}) = 412 (260), 510 (20.7), 547 (6.27), 584 (7.67), 635 (1.51).

1.13 Synthesis of 5,10,15,20-tetrakis[4-(*β*-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]-2,3-[methano(*N*-methyl)iminomethano]chlorin (G-chlorin)

The same procedure as that used for the preparation of Mal$_3$-chlorin was applied to AcG-chlorin (49 mg, 20 μmol) and the formation of the target product was confirmed by TLC (ODS plate; methanol, R_f = 0.87) to give G-chlorin as a green solid (21 mg, 60 %).

1.14 Synthesis of 5,10,15,20-tetrakis[4-(*β*-D-glucopyranosylthio)-2,3,5,6-tetrafluorophenyl]porphyrin (G-porphyrin)

The same procedure as that used for the preparation of Mal$_3$-chlorin was applied to AcG-porphyrin (40 mg, 17 μmol) and the formation of the target product was confirmed by TLC (ODS plate; MeOH, R_f = 0.95) to give G-porphyrin as a brownish-red solid (29 mg, 70 %).

2. Spectral Data

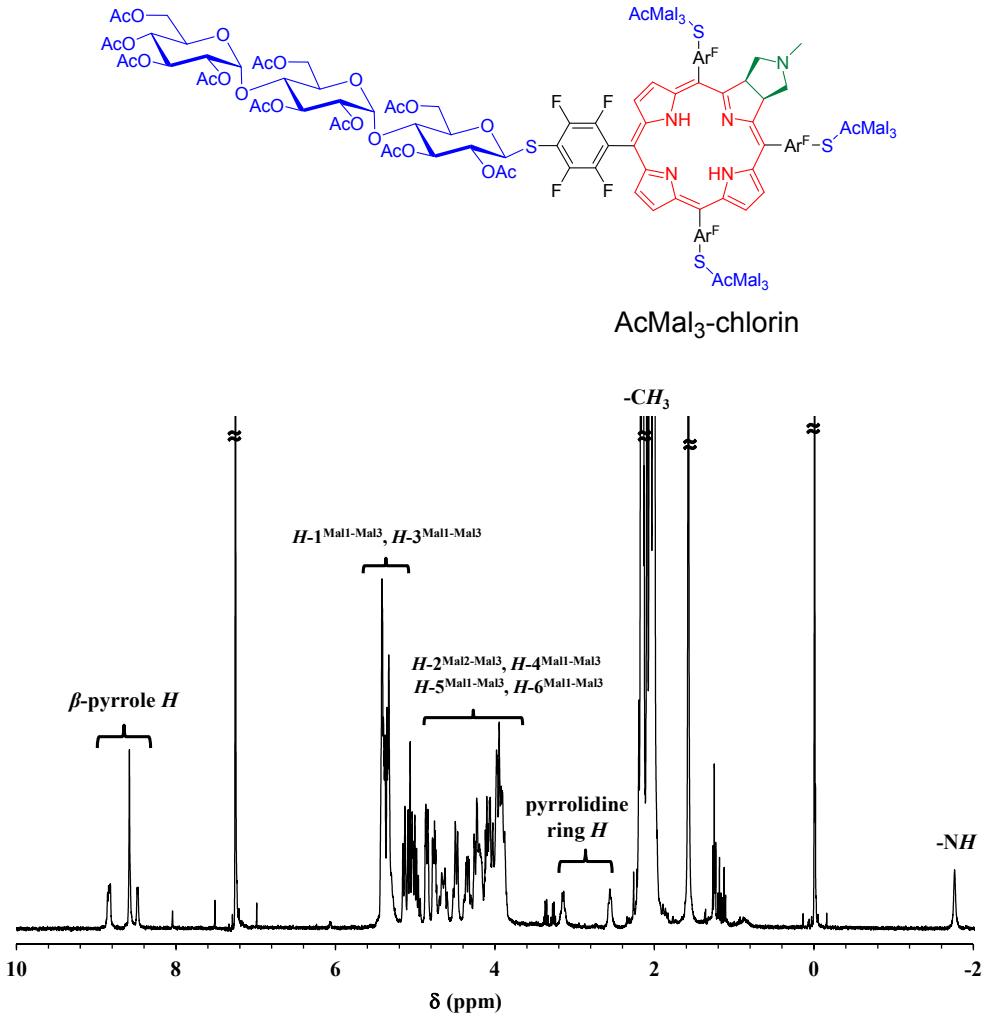


Fig. S1-1. ^{1}H-NMR spectrum of AcMal$_3$-chlorin in CDCl$_3$.

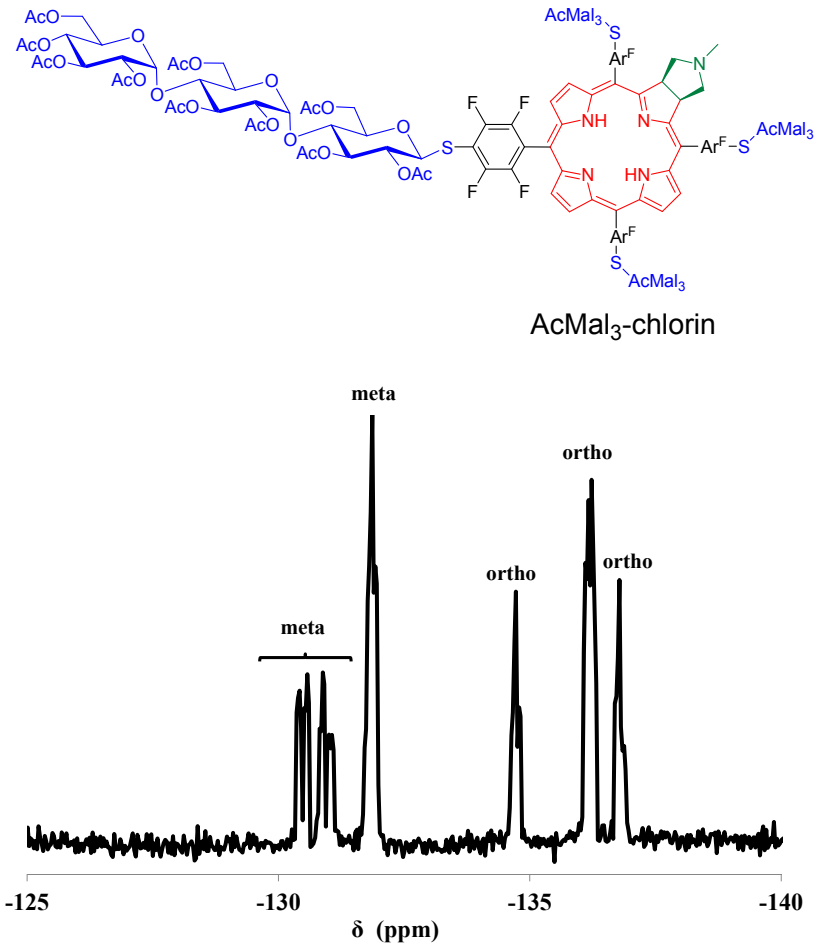


Fig. S1-2. ^{19}F-NMR spectrum of AcMal$_3$-chlorin in CDCl$_3$.

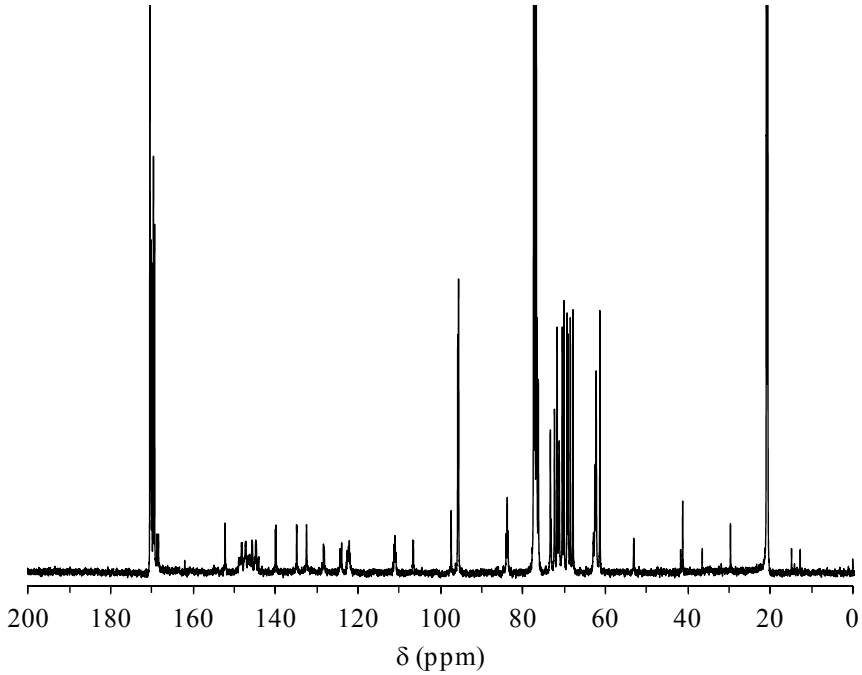


Fig. S1-3. ^{13}C-NMR spectrum of AcMal$_3$-chlorin in CDCl$_3$.

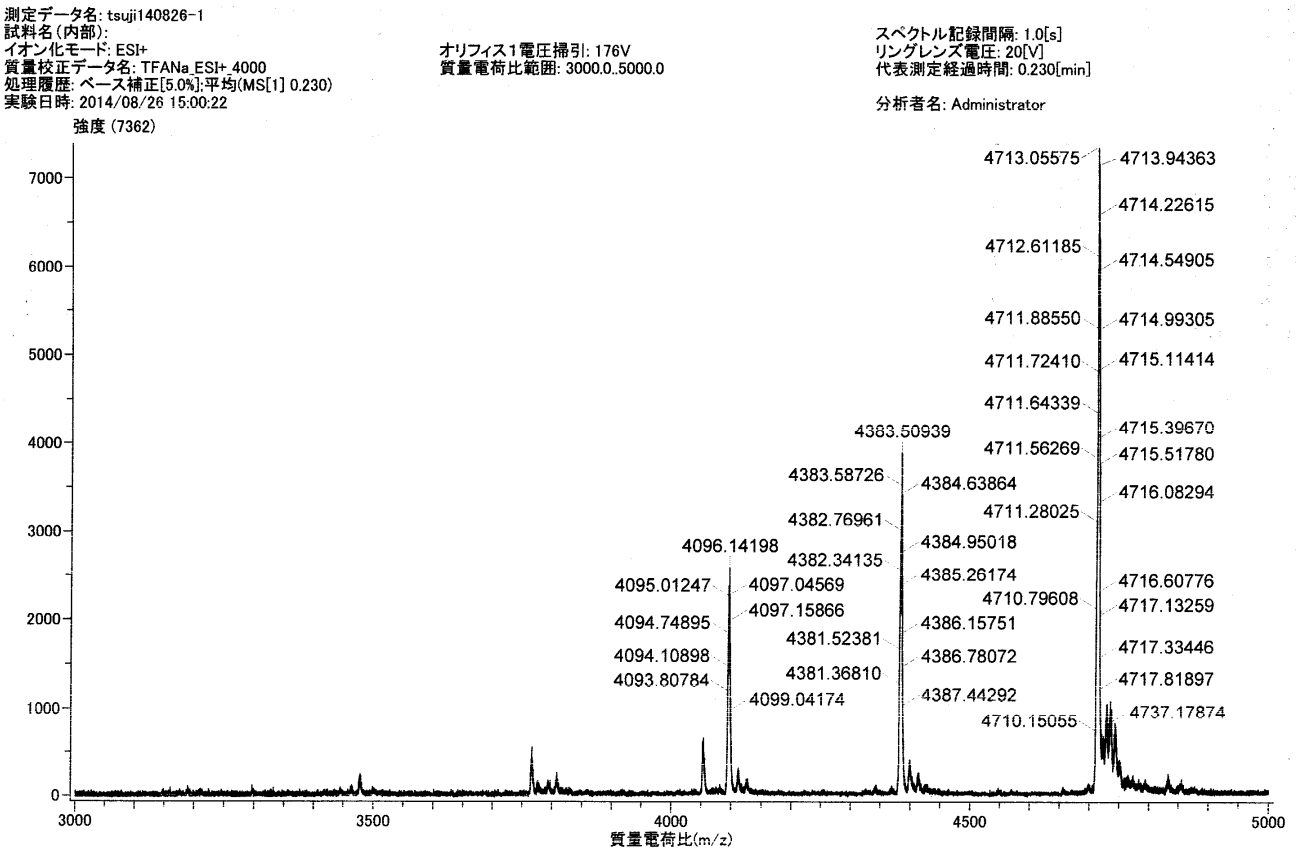


Fig. S1-4. ESI-MS spectrum of $AcMal_3$-chlorin.

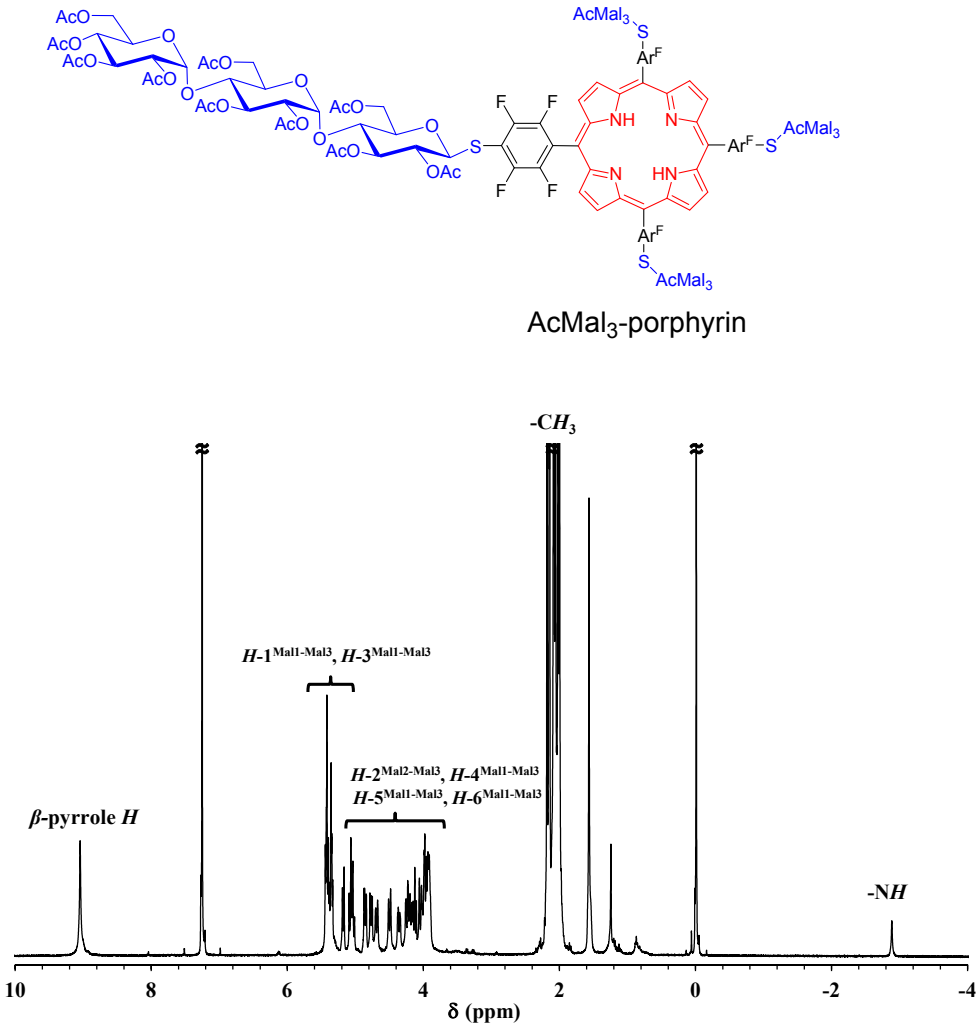


Fig. S2-1. ^{1}H-NMR spectrum of AcMal$_3$-porphyrin in CDCl$_3$.

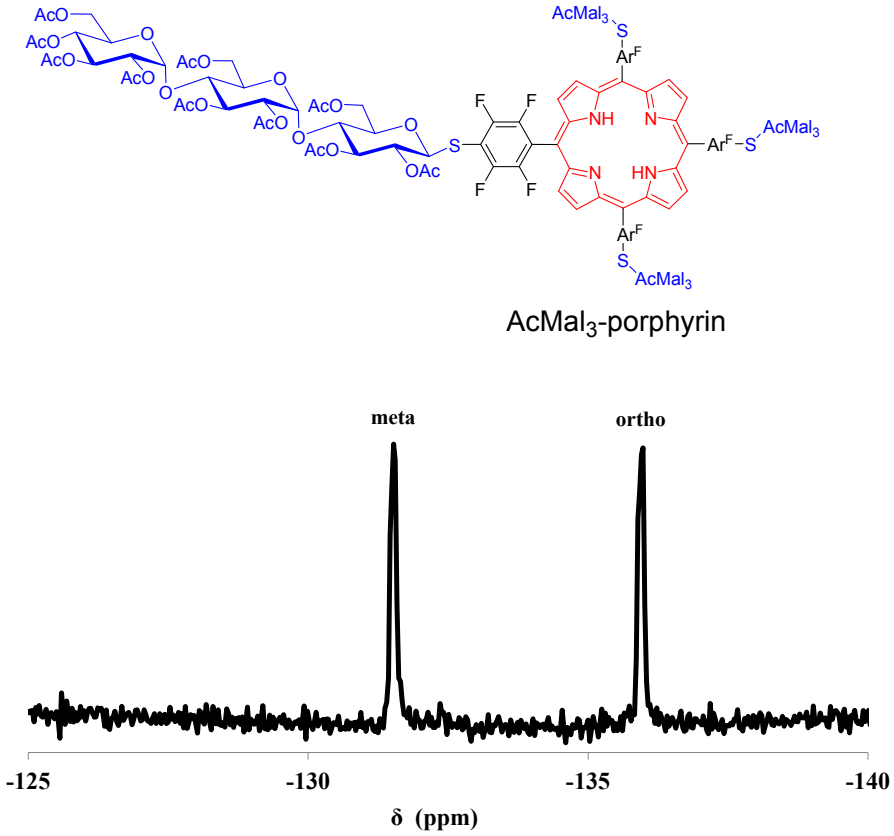


Fig. S2-2. ^{19}F-NMR spectrum of AcMal$_3$-porphyrin in CDCl$_3$.

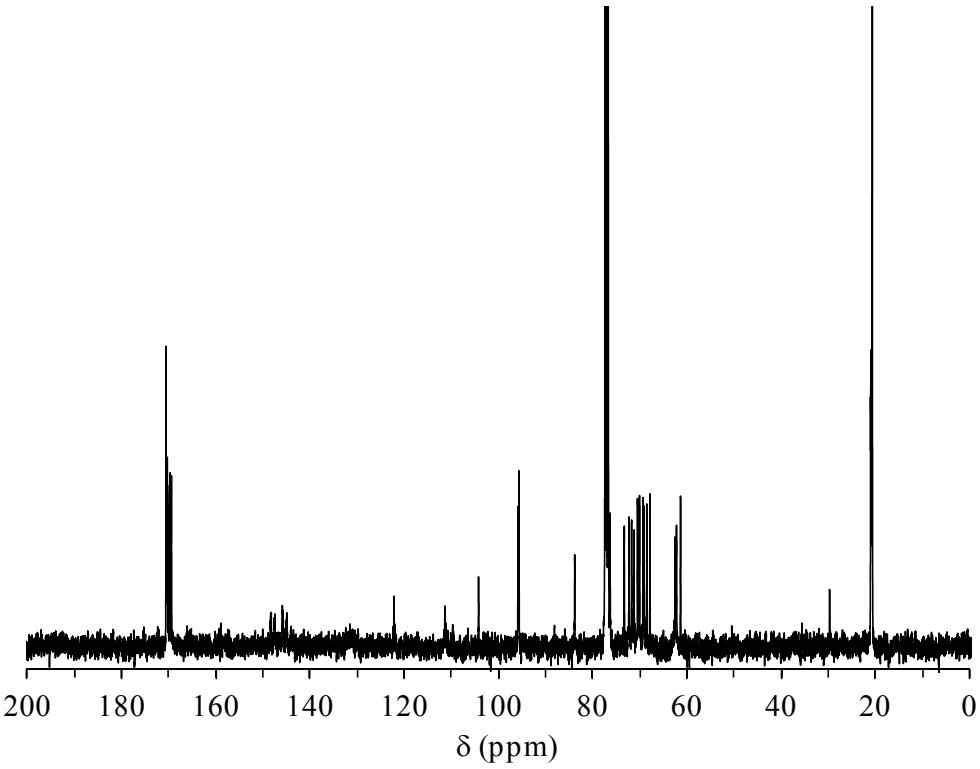


Fig. S2-3. ^{13}C-NMR spectrum of AcMal$_3$-porphyrin in $CDCl_3$.

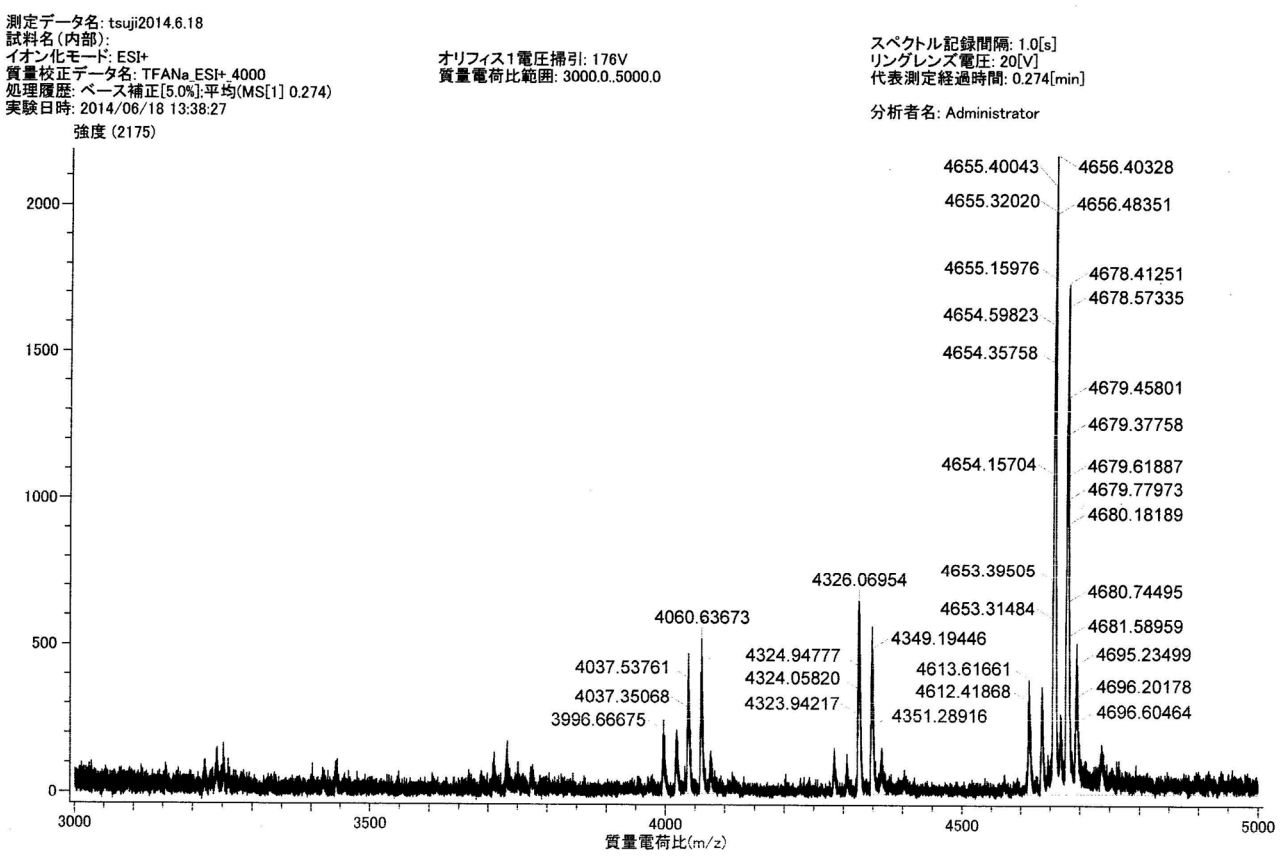


Fig. S2-4. ESI-MS spectrum of $AcMal_3$-porphyrin in $CDCl_3$.

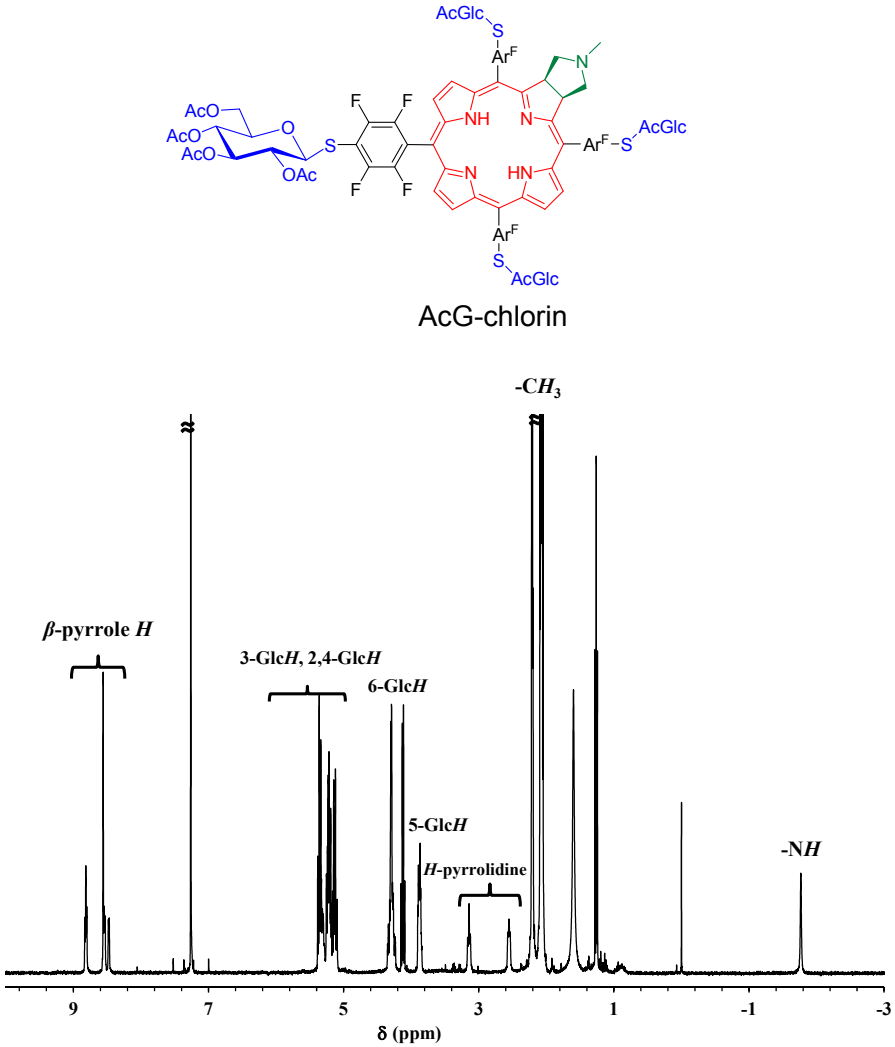


Fig. S3. ^{1}H-NMR spectrum of AcG-chlorin in $CDCl_3$.

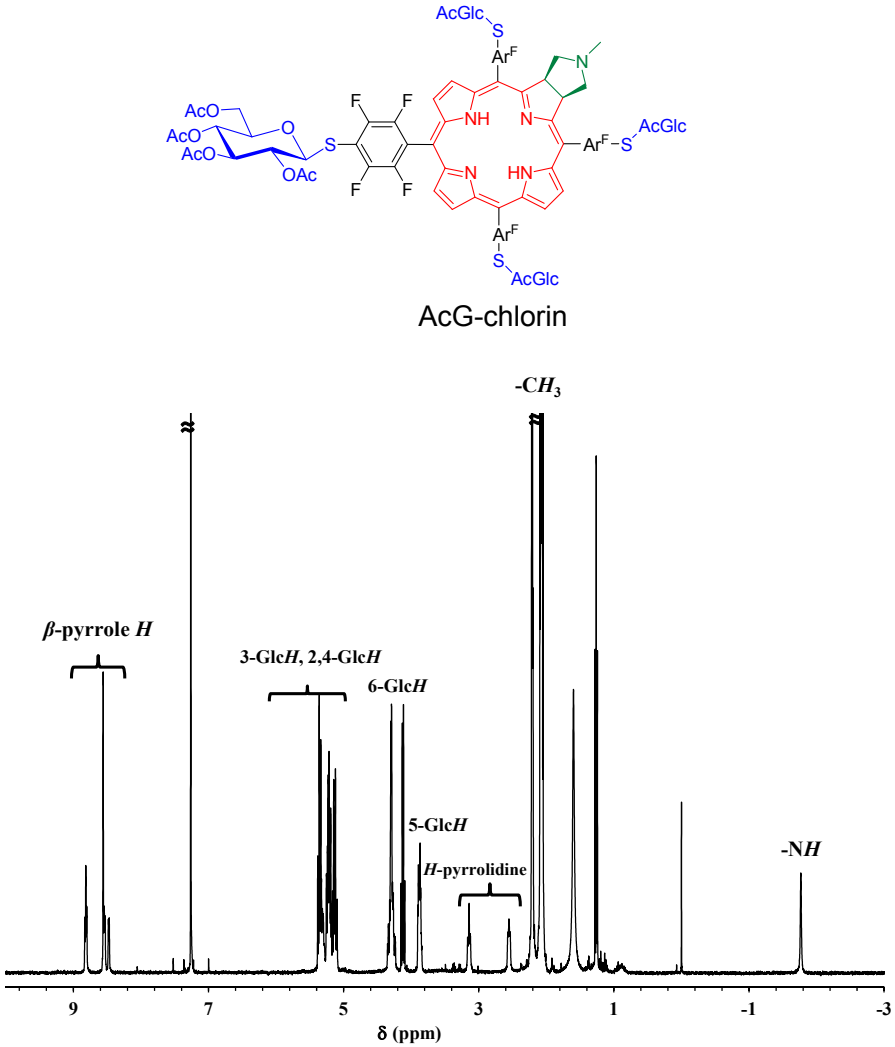
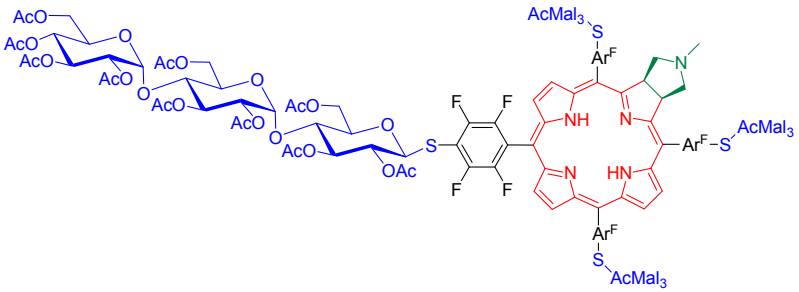
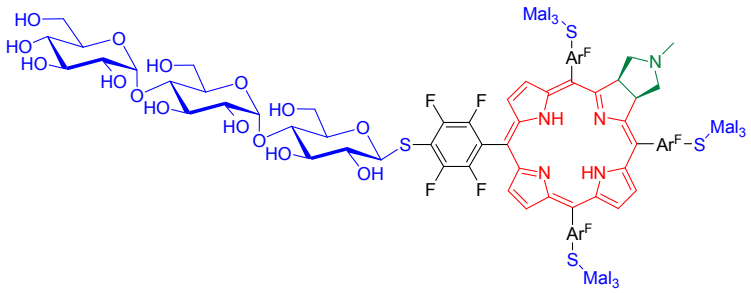


Fig. S4. ^{1}H-NMR spectrum of AcG-porphyrin in $CDCl_3$.





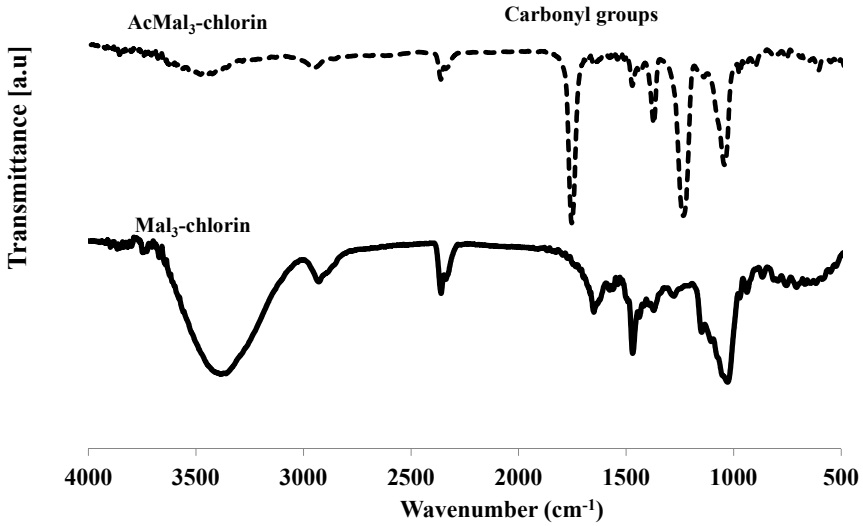
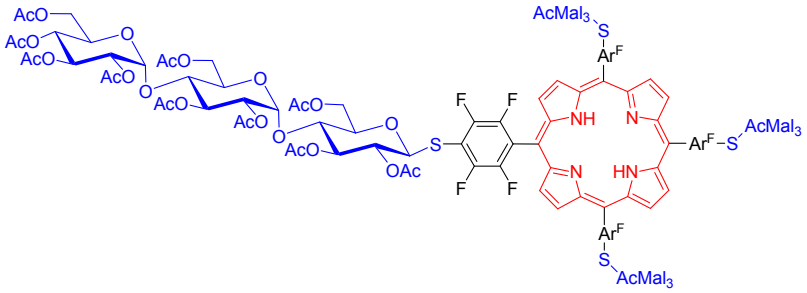
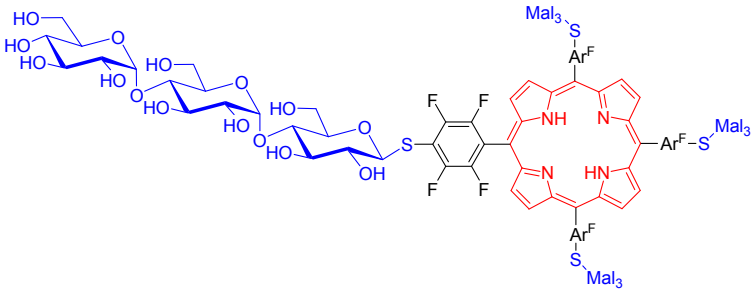


Fig. S5. IR spectra of AcMal$_3$-chlorin (dashed line) and Mal$_3$-chlorin (solid line).





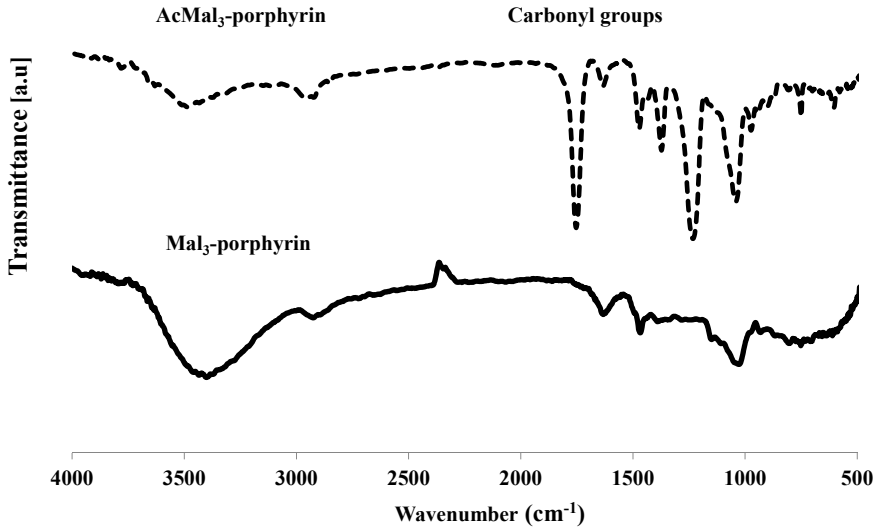


Fig. S6. IR spectra of AcMal3-porphyrin (dashed line) and Mal3-porphyrin (solid line).

3. UV-vis Absorption Properties

Table S1. Summary for UV-vis absorption property

Sample	Solvent	λ_{max}/nm ($\varepsilon \times 10^{-3}$/M^{-1} cm^{-1})				
		Soret	Q bands			
Mal_3-chlorin	DMSO	415 (178)	507 (17.2)	537 (6.92)	598 (6.64)	652 (33.4)
Mal_3-chlorin	Water	407 (148)	507 (16.5)	536 (7.17)	597 (5.58)	649 (34.6)
Mal_3-chlorin	PBS	407 (147)	507 (16.7)	534 (6.87)	598 (5.63)	651 (35.9)
TFPC	DMSO	407 (169)	503 (15.6)	536 (2.78)	597 (5.05)	652 (41.6)
Mal_3-porphyrin	DMSO	415 (290)	507 (22.9)	539 (3.68)	581 (7.57)	636 (0.99)
Mal_3- porphyrin	Water	412 (260)	510 (20.7)	547 (6.27)	584 (7.67)	644 (1.51)
TFPP	DMSO	411 (255)	505 (20.8)	538 (2.29)	579 (6.29)	632 (0.82)

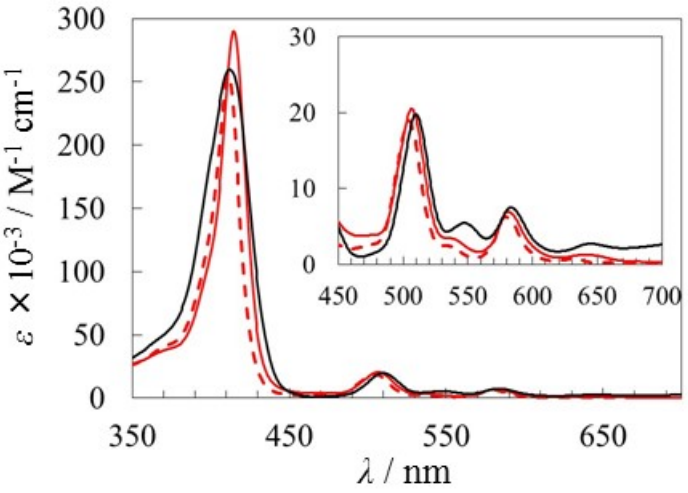


Fig. S7. UV-vis spectra of Mal_3-porphyrin in DMSO (red solid line) and TFPP in DMSO (red dashed line) and Mal_3-porphyrin in water (black solid line).

4. Quantum Yield of $^1O_2$ Generation

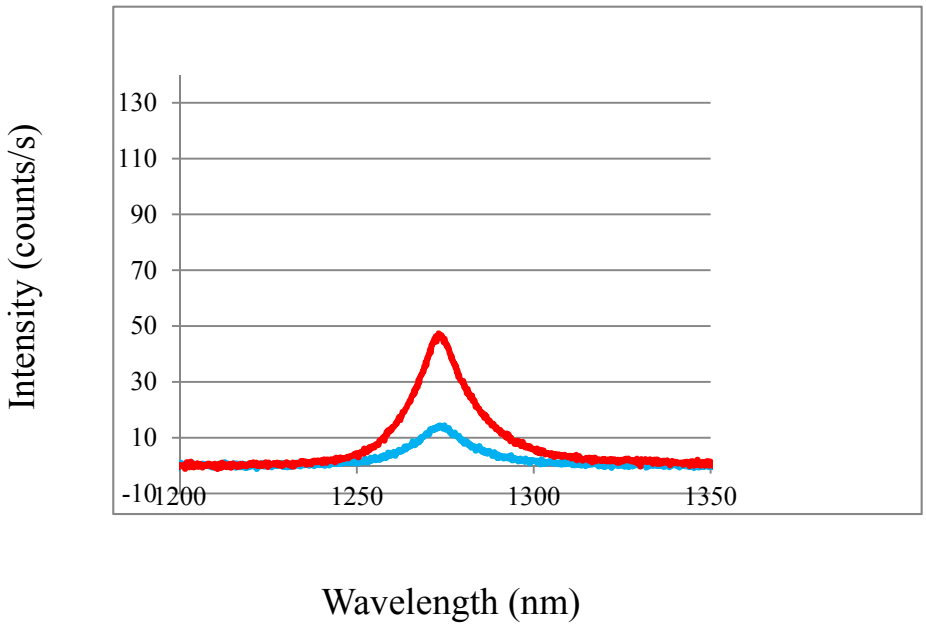


Fig. S8. Emission spectra of $^1O_2$ at 1280 nm generated from NPe6 (blue) and Mal$_3$-chlorin (red) by laser irradiation (532nm) in oxygen-saturated D_2O.

Table S2. The quantum yields of $^1O_2$ from NPe6 and Mal$_3$-chlorin by laser irradiation (532 nm) in oxygen-saturated D_2O

Sample	Molecular weight	Intensity (counts/s)	Quantum yield
NPe6	799.69	14.40	0.08
Mal$_3$-chlorin	3033.62	47.37	0.28

5. Fluorescence Microscopy Measurements

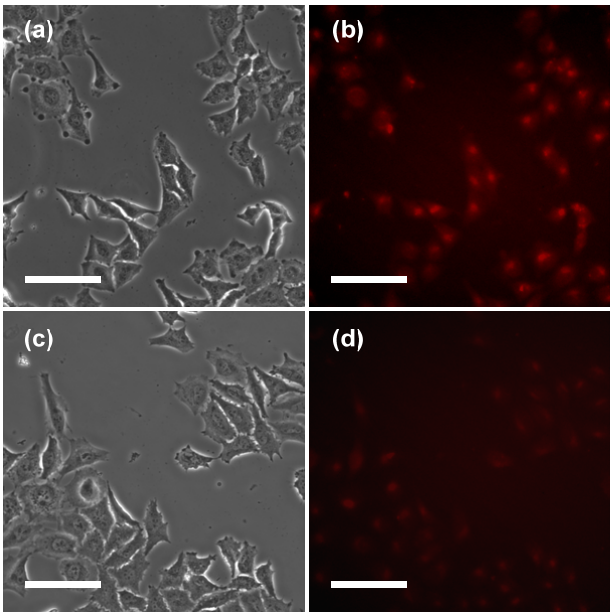


Fig. S9. Phase contrast (a and c) and fluorescence (b and d) images of the HeLa cells after being treated with (a and b) Mal_3-chlorin and (c and d) Mal_3-porphyrin for 24 h at 37 ºC. The scale bar represents 100 μm.

6. References

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