Electronic Supplementary Information

D-Mannose-appended 5,15-diazaporphyrin for photodynamic therapy

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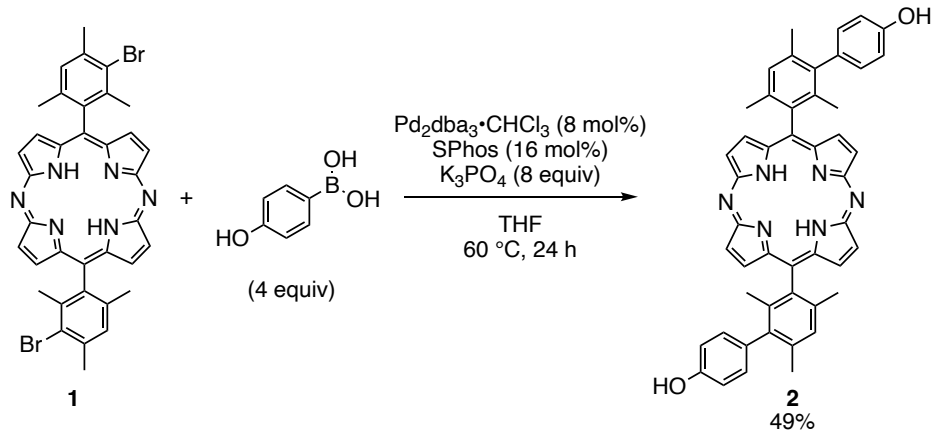
1. Instrumentation and Materials

^{1}H NMR (500 MHz) and ^{13}C NMR (126 MHz) spectra were recorded on a Bruker AVANCE III HD spectrometer. ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded on a Bruker AVANCE NEO spectrometer. Chemical shifts were reported as the delta scale in ppm relative to $CHCl_3$ (δ = 7.26 ppm) for ^{1}H NMR and $CDCl_3$ (δ = 77.16 ppm) for ^{13}C NMR. UV/Vis/NIR absorption spectra were recorded on a JASCO V 670 spectrometer. High-resolution electrospray ionization time-of-flight (ESI-TOF) mass spectra were taken on a Bruker micrOTOF instrument. High-resolution matrix assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectra were taken on a Bruker autoflex max using a positive ionization mode. *N,N*-Dimethylformamide (NMP), tetrahydrofuran (THF) and $CHCl_3$ was purchased from FUJIFILM Wako Pure Chemical Corporation as a super dehydrated grade. Dry CH_2Cl_2 and dry toluene was prepared by distillation from CaH_2. 10,20-Di(3-bromo-2,4,6-trimethylphenyl)-5,15-diazaporphyrin **1** was prepared according to the literature.[1] Unless otherwise noted, materials obtained from commercial suppliers were used without further purification.

2. Synthetic Procedures and Compound Data

10,20-Di(3-(4-hydroxyl)phenyl-2,4,6-trimethylphenyl)-5,15-diazaporphyrin 2.

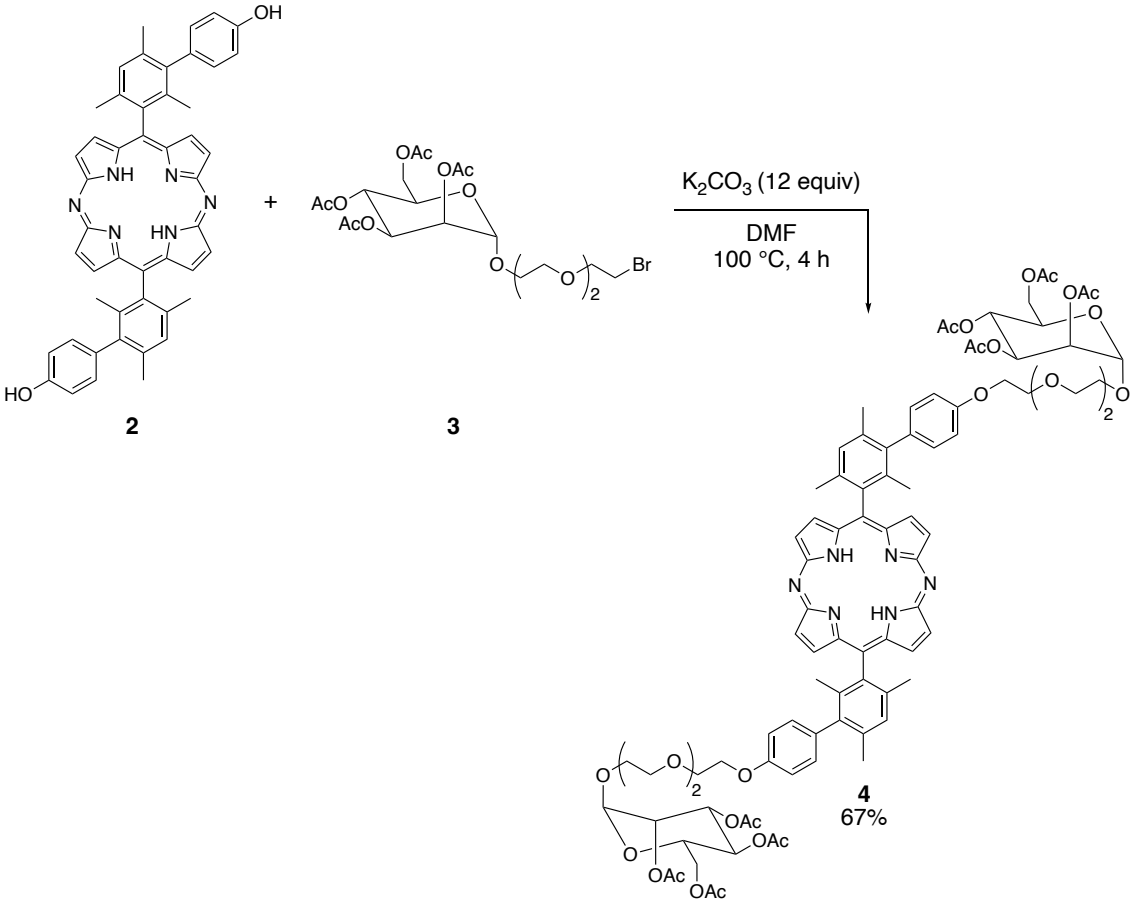


A Schlenk tube containing **1** (21.2 mg, 30 μmol), $Pd_2(dba)_3$•$CHCl_3$ (2.48 mg, 2.4 μmol), 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (SPhos) (1.97 mg, 4.8 μmol), K_3PO_4 (50.9 mg, 240 μmol), and 4-hydroxyphenylboronic acid (16.6 mg, 120 μmol) was filled with N_2, and then charged with dry THF (2.5 mL). The resulting solution was stirred at 60 °C for 24 h. The product was extracted with AcOEt, washed with brine, and dried over Na_2SO_4. After removal of the solvent *in vacuo*, the residue was purified by silica gel chromatography eluting with

CH_2Cl_2/AcOEt (5/1). After removal of the solvent *in vacuo*, recrystallization from AcOEt/*n*-hexane afforded **2** (10.8 mg, 14.7 μmol, 49%).

^{1}H NMR (300 MHz, acetone-d_6, 253 K): δ = 9.33 (d, J = 4.5 Hz, 4H, β), 8.98 (d, J = 4.8 Hz, 4H, β), 8.36 (s, 2H, OH), 7.50 (s, 2H, Mes), 7.32–7.29 (m, 4H, Ph), 7.02–6.98 (m, 4H, Ph), 2.38 (s, 6H, Mes), 1.88 (s, 6H, Mes), 1.58 (s, 6H, Mes), –2.55 (s, 2H, NH) ppm; ^{13}C{^{1}H} NMR (126 MHz, acetone-d_6, 298 K): δ = 157.2, 140.8, 138.6, 138.4, 138.1, 137.0, 134.3, 133.5, 133.0, 131.4, 129.5, 122.3, 116.4, 116.3, 21.8, 21.6, 20.6 ppm (Some signals due to aromatic protons are too broad to be observed because of the amine-imine tautomerization); MS (MALDI-TOF): $[M+H]^+$ Calcd for $C_{48}H_{40}N_6O_2$ 733.3286; Found 733.2975.

Bis(2,3,4,6-Tetra-O-acetyl-D-mannopyranose)-appended 5,15-diazaporphyrin 4.

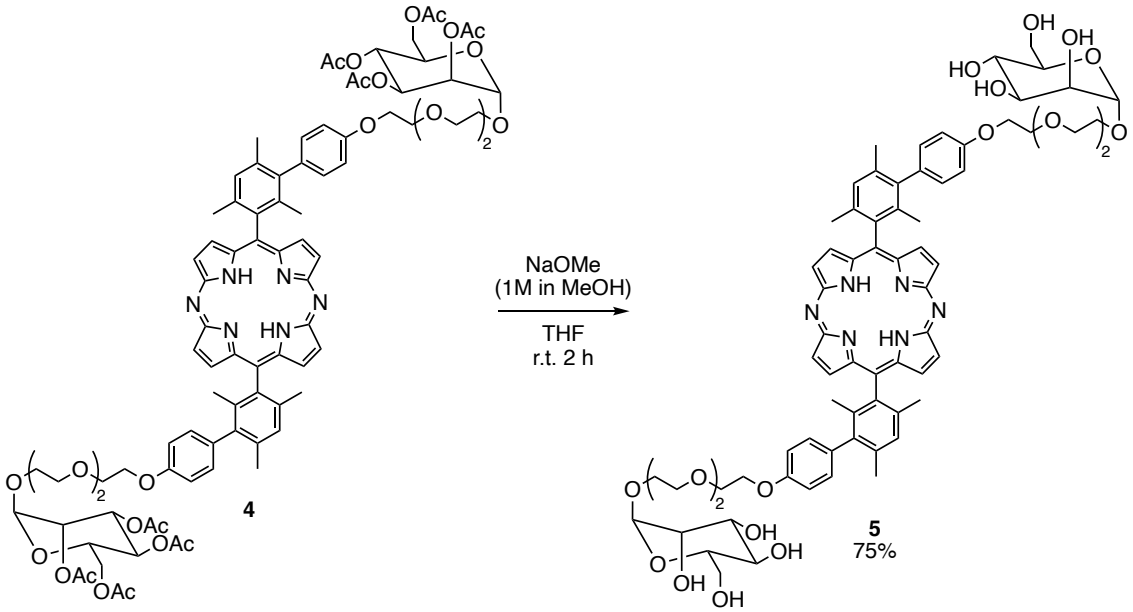


A Schlenk tube containing **2** (22.0 mg, 30 μmol) and K_2CO_3 (49.7 mg, 360 μmol) was filled with N_2, and then charged with dry DMF (2.0 mL). To the tube, a solution of **3** (86.5 mg, 159 μmol) in DMF (1.0 mL) was added. After stirring at 100 °C for 4 h, the product was extracted with AcOEt, washed with brine, and dried over Na_2SO_4. After removal of the solvent *in vacuo*, the residue was purified by silica gel chromatography eluting with CH_2Cl_2/AcOEt (3/1). After removal of the solvent *in vacuo*, recrystallization from AcOEt/*n*-hexane provided **4** (33.3 mg,

20.1 μmol, 67%).

^{1}H NMR (500 MHz, $CDCl_3$, 253 K): δ = 9.29 (d, J = 5.0 Hz, 4H, β), 8.94 (d, J = 4.5 Hz, 4H, β), 7.40 (s, 2H, Mes), 7.40–7.31 (m, 4H, Ph), 7.06–7.03 (m, 4H, Ph), 5.36–5.34 (m, 2H, mannose), 5.29–5.25 (m, 4H, mannose), 4.87 (s, 2H, anomeric), 4.30–4.26 (m, 2H, mannose), 4.20–4.17 (m, 4H, TEG), 4.10–4.04 (m, 4H, mannose), 3.90–3.88 (m, 4H, TEG), 3.83–3.78 (m, 2H, TEG), 3.74–3.73 (m, 4H, TEG), 3.69–3.66 (m, 10H, TEG), 2.35 (s, 6H, Mes), 2.13 (s, 6H, Ac), 2.08 (s, 6H, Ac), 2.00 (s, 6H, Ac), 1.96 (s, 6H, Ac), 1.86 (s, 6H, Mes), 1.53 (s, 6H, Mes), –2.52 (s, 2H, NH) ppm (Some signals due to mesityl, phenyl, and acetyl groups are split because of the presence of rotamers due to the mesityl groups); ^{13}C{^{1}H} NMR (126 MHz, $CDCl_3$, 298 K): δ = 170.8, 170.2, 170.0, 169.9, 157.8, 139.5, 138.2, 138.0, 137.4, 136.2, 133.7, 133.0, 130.7, 128.7, 114.8, 97.8, 71.0, 70.9, 70.2, 70.0, 69.7, 69.2, 68.6, 67.5, 66.3, 62.6, 21.9, 21.5, 21.0, 20.9, 20.8, 20.5 ppm (Some signals were overlapped.); HRMS (ESI-TOF): $[M+H]^+$ Calcd for $C_{88}H_{100}N_6O_{26}$ 1657.6760; Found 1657.6769.

Bis(D-mannopyranose)-appended 5,15-diazaporphyrin 5.



A Schlenk tube containing **4** (16.6 mg, 10 μmol) was charged with dry THF (2.0 mL). To the tube, a THF solution of sodium methoxide (1.0 M, 0.2 mL) was added. After stirring at room temperature for 2 h, the product was extracted with THF, washed with brine, and dried over Na_2SO_4. After removal of the solvent *in vacuo*, recrystallization from THF/*n*-hexane afforded **5** (9.9 mg, 7.5 μmol, 75%).

^{1}H NMR (500 MHz, DMSO-d_6, 253 K): 9.41 (d, J = 4.5 Hz, 4H, β), 8.93 (d, J = 4.5 Hz, 4H, β), 7.49 (s, 2H, Mes), 7.35 (d, J = 9.0 Hz, 4H, Ph), 7.07 (d, J = 9.0 Hz, 4H, Ph), 4.71–4.68 (m, 4H, OH), 4.62 (d, J = 1.5 Hz, 2H, mannose), 4.55 (d, J = 5.5 Hz, 2H, OH), 4.42–4.40 (m, 2H,

OH), 4.12–4.11 (m, 4H, mannose), 3.76–3.74 (m, 4H, mannose), 3.69–3.40 (m, 28H, mannose + TEG), 2.30 (s, 6H, Mes), 1.80 (s, 6H, Mes), 1.44 (s, 6H, Mes), –2.70 (s, 2H, NH)ppm (Some signals due to mesityl groups, phenylene groups and acetyl groups were split because of rotate isomer of mesityl group.); $^{13}C\{^{1}H\}$ NMR (126 MHz, DMSO-d_6, 298 K): δ = 157.3, 139.2, 137.3, 136.8, 136.6, 135.5, 133.6, 132.8, 132.6, 130.5, 128.6, 120.9, 114.6, 99.9, 73.9, 72.3, 70.9, 70.3, 69.9, 69.8, 69.8, 69.5, 69.0, 67.0, 65.7, 61.3, 60.2, 21.2, 21.0, 20.2 ppm (Some signals due to aromatic protons are too broad to be observed because of the amine-imine tautomerization); HRMS (ESI-TOF): $[M–H]^-$ Calcd for $C_{72}H_{84}N_6O_{18}$ 1319.5758; Found 1319.5768. UV-Vis (H_2O/DMSO(5 %)) λ_{max} (ε): 395 (61200), 490 (3700), 510 (4600), 547 (16500), 580 (5500), 632 (22300), 663 (3600).

3. NMR Spectra

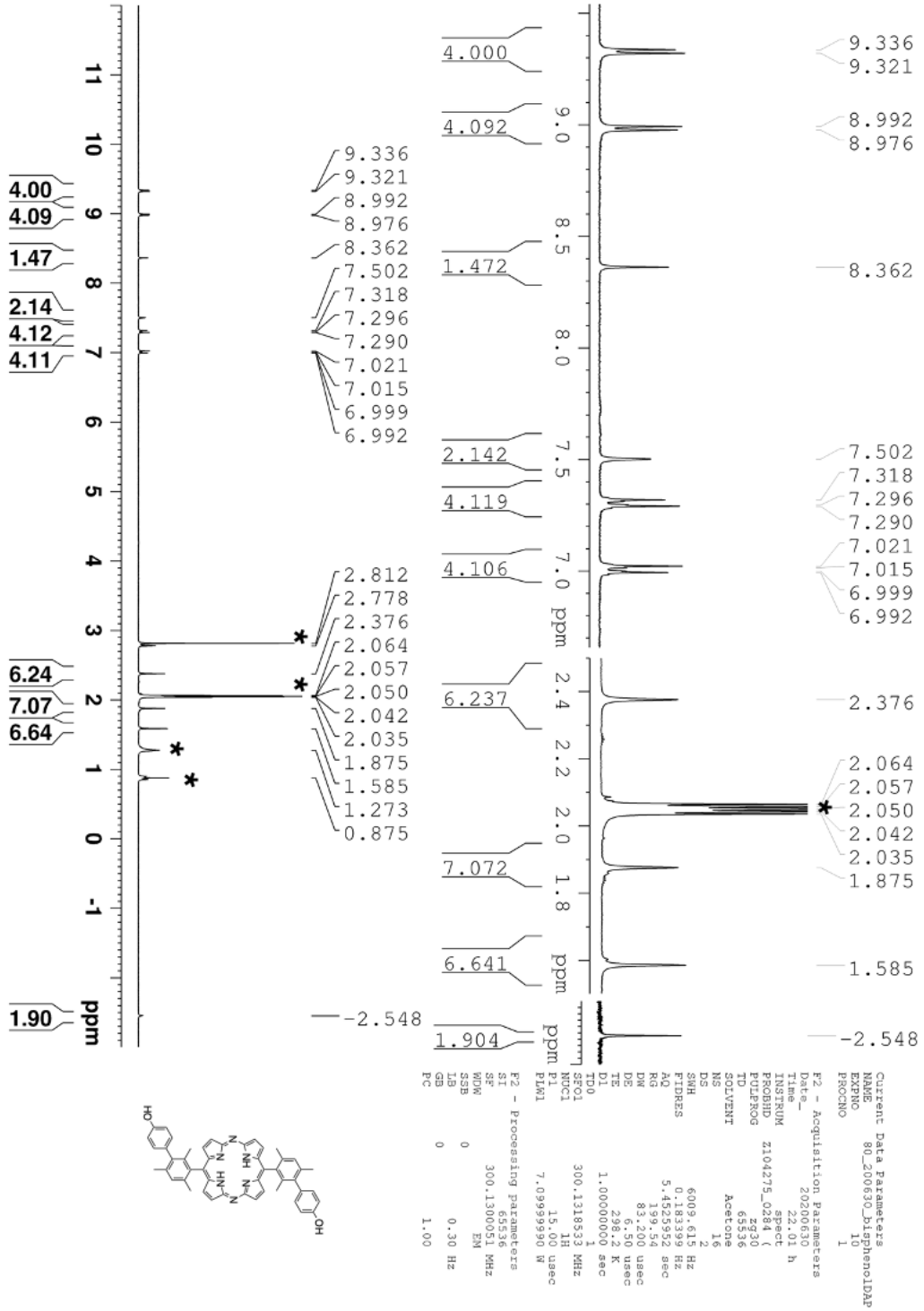


Figure S1 ^{1}H NMR spectra spectrum of **2** in acetone-d_6 at 25 °C.

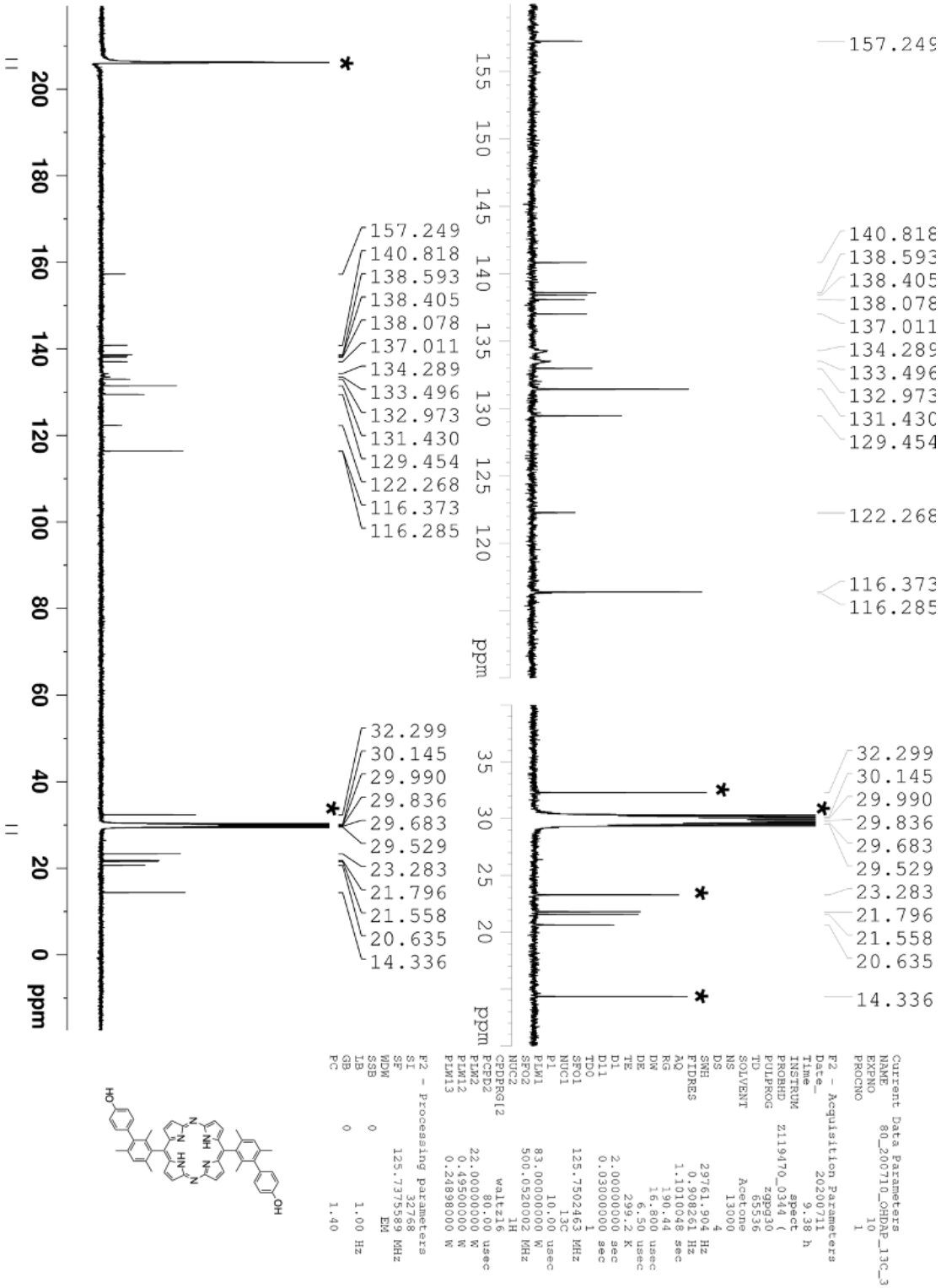


Figure S2 $^{13}C\{^{1}H\}$ NMR spectra spectrum of **2** in acetone-d_6 at 25 °C.

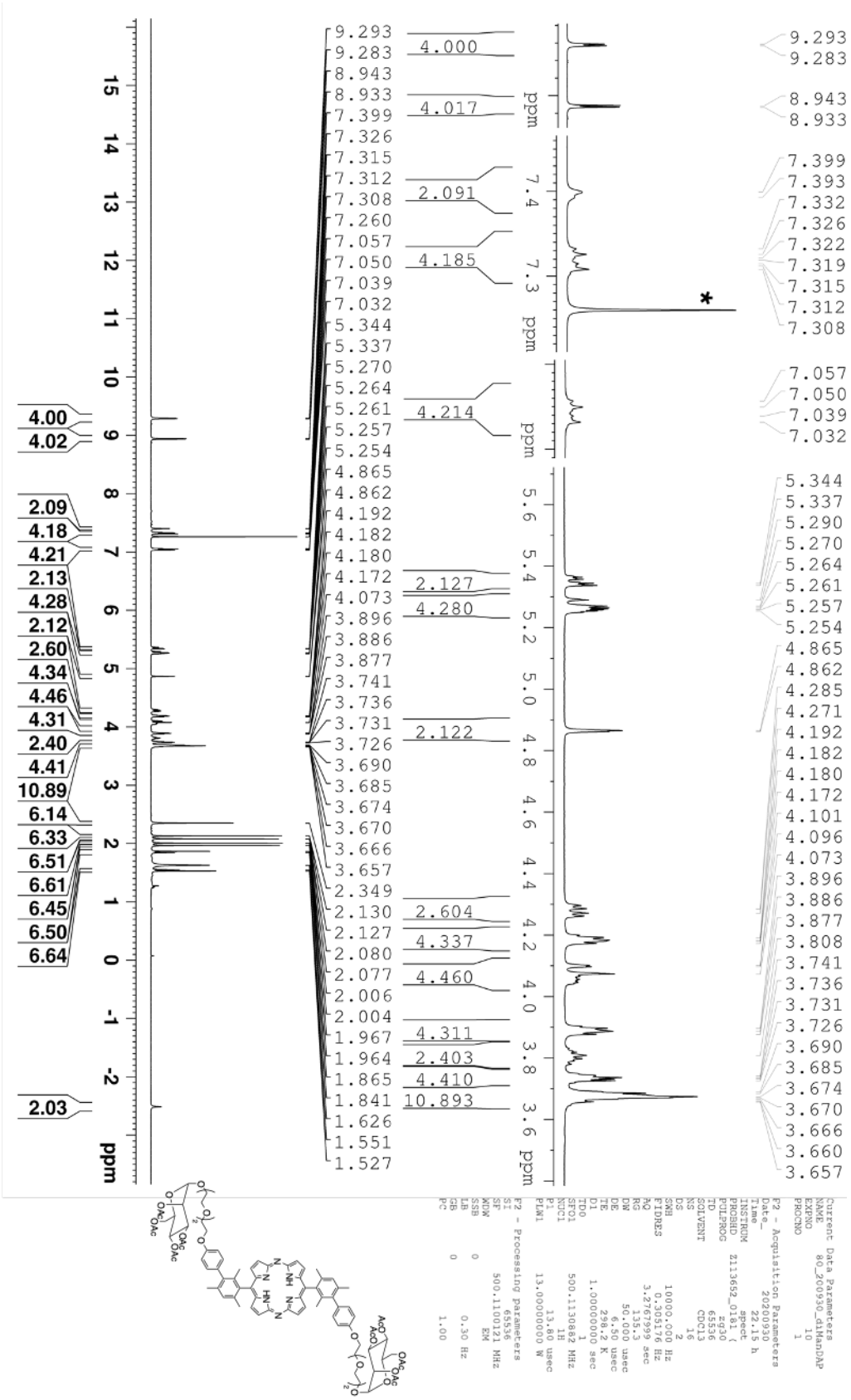


Figure S3 ^{1}H NMR spectra spectrum of **4** in $CDCl_3$ at 25 °C.

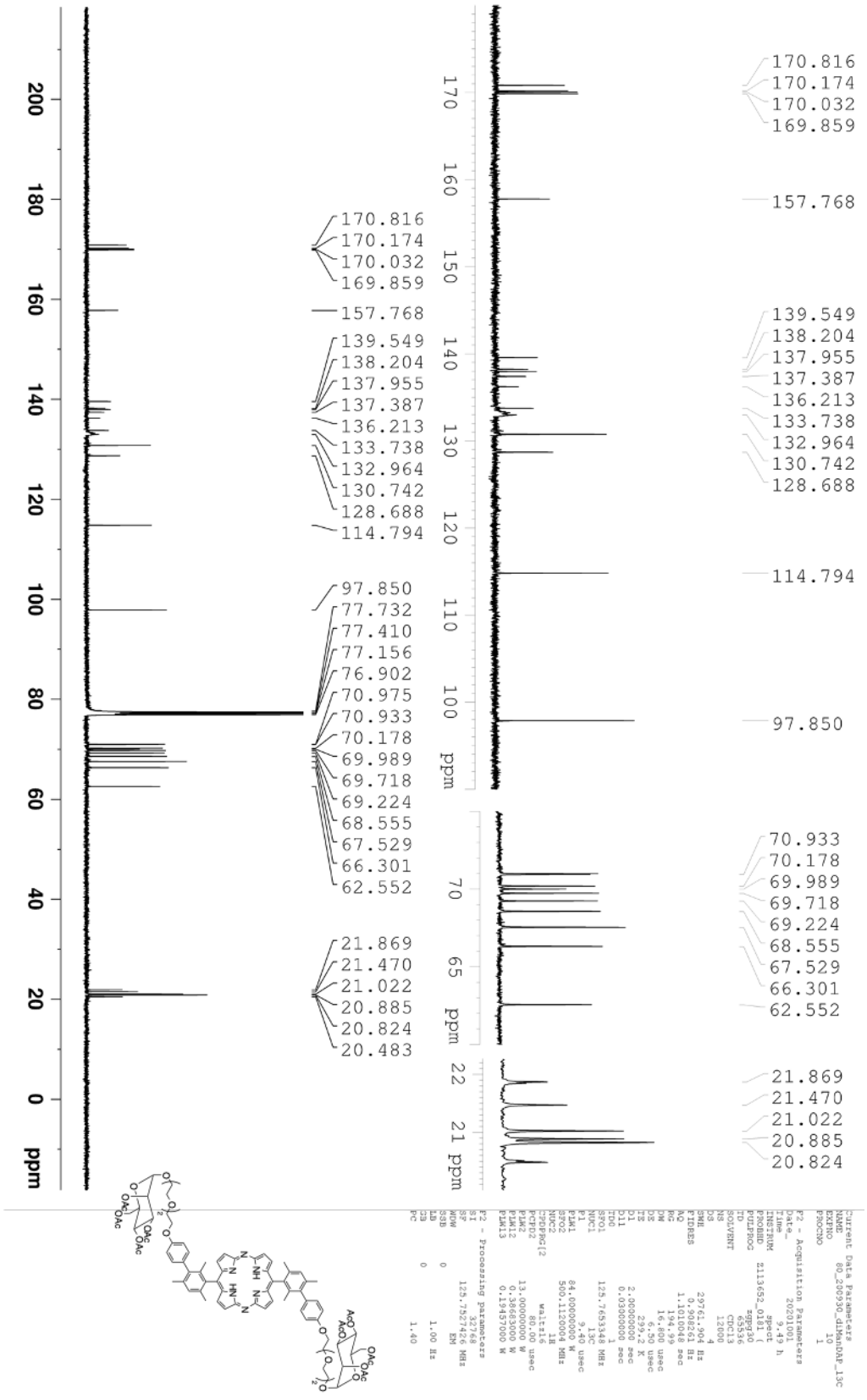


Figure S4 ^{13}C NMR spectra spectrum of **4** in $CDCl_3$ at 25 °C.

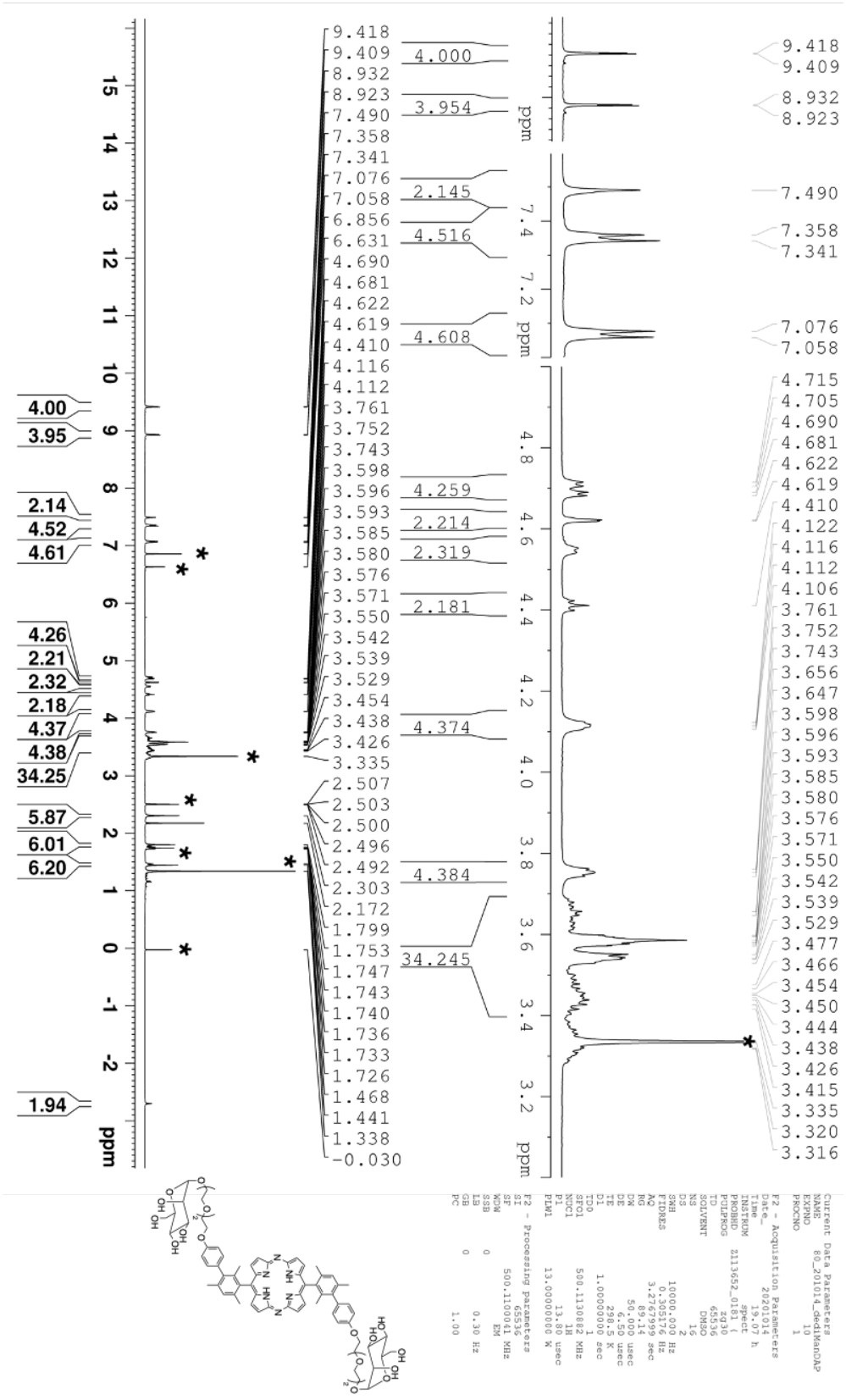


Figure S5 ^{1}H NMR spectra spectrum of **5** in DMSO-d_6 at 25 °C.

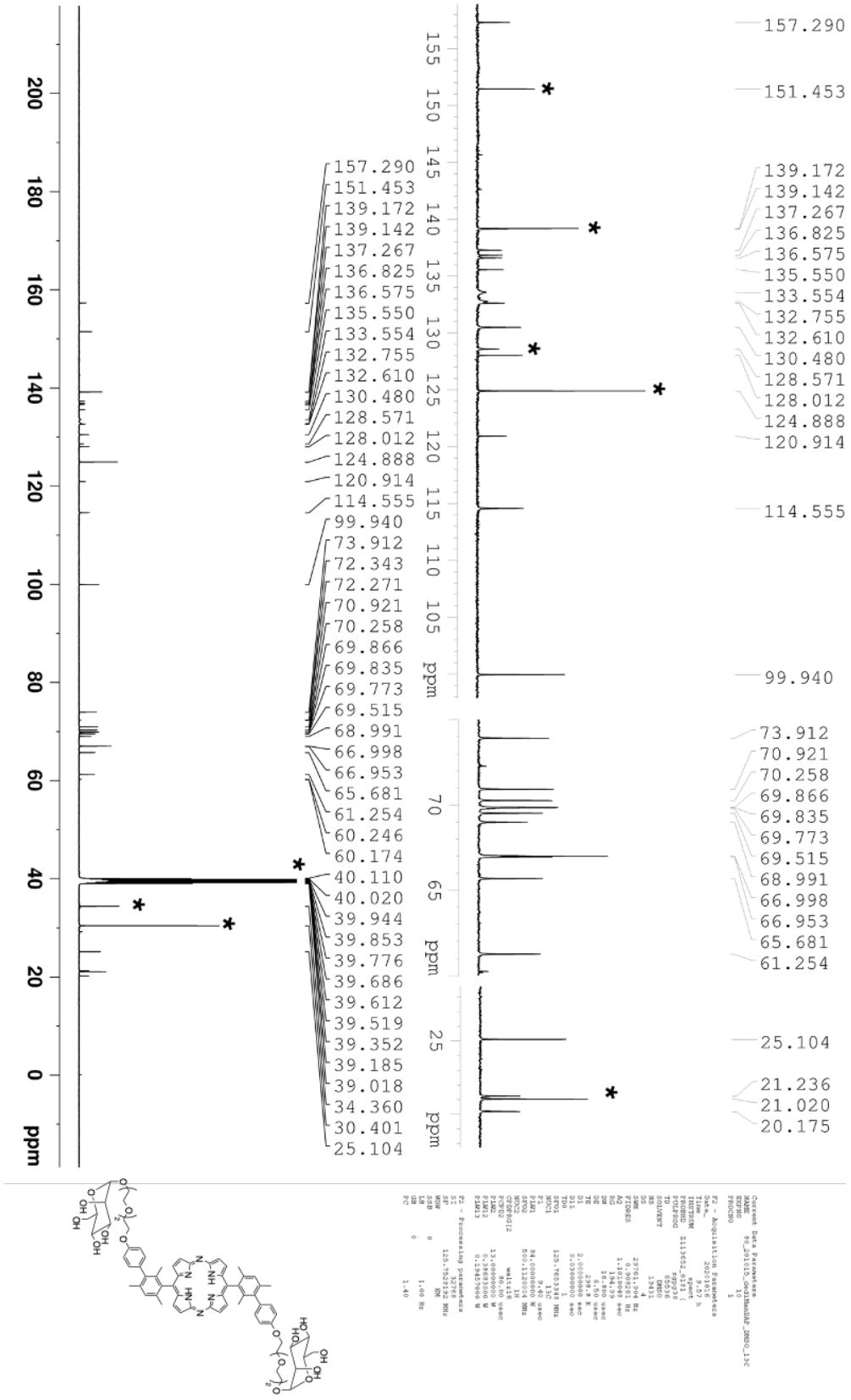


Figure S6 $^{13}C\{^{1}H\}$ NMR spectra spectrum of **5** in DMSO-d_6 at 25 °C.

4. Singet Oxygen Generation Quantum Yield

A stock solution of **DAP** at 1.25 × 10^{-5} M was prepared in toluene. A stock solution of compound **5** was prepared in toluene and MeOH (2%). A stock solution of 9,10-dimethylanthracene (**DMA**) at 1 × 10^{-3} M was prepared in aerated toluene. To get sample solutions, 200 µL of the stock solution of **DMA**, 40 µL of the stock solution of **DAP** or **5** (or 40 µL of toluene for the control experiment), and 1760 µL of toluene were mixed. Final concentrations of the sensitizer and **DMA** were 0.25 and 100 µM, respectively. The obtained solution was placed in a 10 mm quartz cuvette (Hellma) and submitted to irradiation for 16 min in a HepatoChem EvoluChem™ PhotoRedOx Box. The EvoluChem™ LED spotlight was used for irradiation, which has a maximum emission centered at 525 nm (relative radiance = 10 mW/cm², see https://www.hepatochem.com/photoreactors-leds-accessories/led-evoluchem/). The UV/Vis absorption spectra showing the absorbance intensity decay of **DMA** were recorded every two minutes with a JASCO V-750 UV/Visible-NIR spectrophotometer.

Plots of $\ln(A_0/A)$ vs. irradiation time (t) were linear and consistent with first order reactions. The slopes allowed the determination of the photodegradation rates k ($\ln([DMA]_0/[DMA]) = kt$). To determine the singlet oxygen quantum yield of **5**, we used the following equation:

$$\frac{\Phi_\Delta . A}{k} \qquad \frac{\Phi_\Delta^r . A^r}{k^r}$$

Where the superscript r stands for a reference, which is **DAP** ($\Phi_\Delta = 0.74$) in toluene.

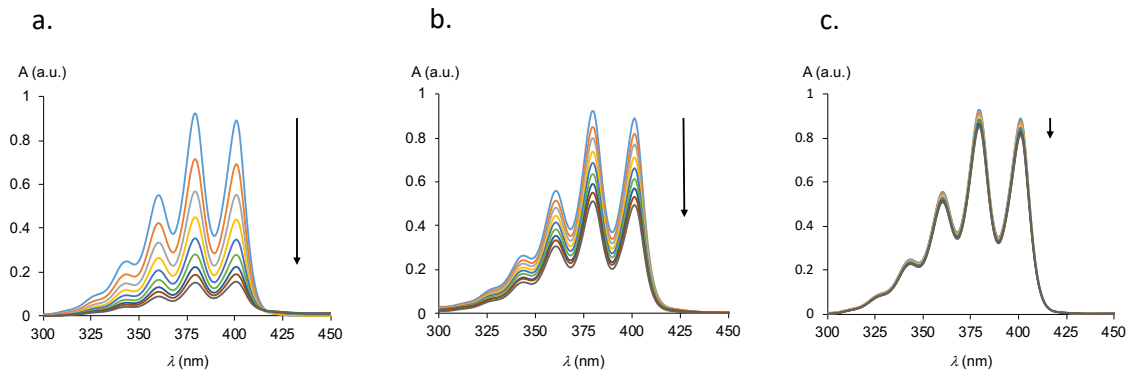


Figure S7 UV/Vis absorption spectra in toluene showing the absorbance intensity decay of **DMA** during 16 min under irradiation with green light (λ_{exc} = 525 nm, LED irradiation) in the presence of (a) **DAP** and (b) **5**. (c) Control experiment was performed without diazaporphyrin sensitizers. Final concentration of the sensitizer and **DMA** were 0.25 and 100 µM, respectively.

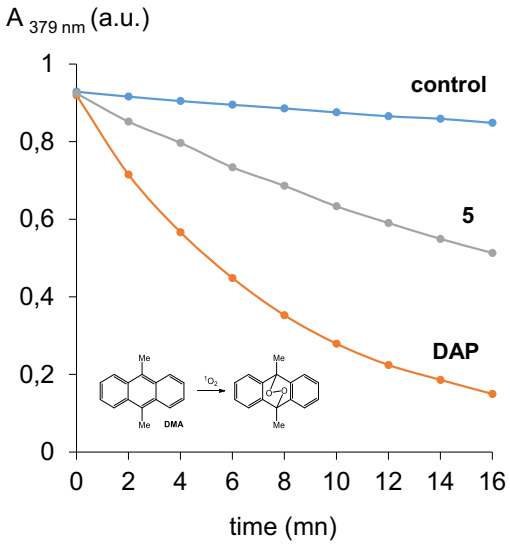


Figure S8 Comparative plots of A $_{379\,nm}$ vs. time (t) demonstrating the absorbance intensity decay of DMA during 16 minutes irradiation with green light (λ_{exc} = 525 nm, LED irradiation).

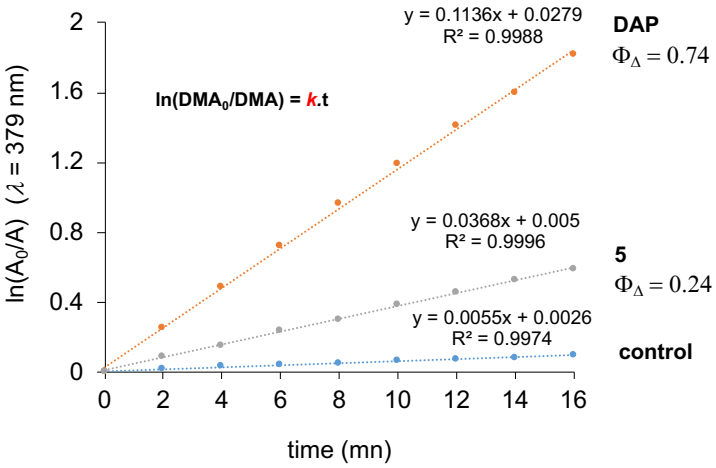


Figure S9 Comparative plots of ln(A_0/A) vs. time (t) demonstrating the photooxidation rates with **DAP** and **5** as photosensitizers and DMA as the $^1O_2$ scavenger. Irradiation was performed with green LED (λ_{exc} = 525 nm). Control experiment was performed without photosensitizers.

5 Biological Studies

Cell culture

Human breast cancer cell line (MDA-MB-231) was purchased from ATCC. Cells were maintained in Dulbecco's Modified Eagle Medium Gibco™ (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% gentamycin. Cells were incubated at 37°C in humidified atmosphere with 5% CO_2.

Cell viability assay

MDA-MB-231 cells were seeded in 96-well plates, in their respective medium. After 24 h, cells were treated with different concentrations of **2**, **4** and **5** ranging from 0 to 5 µM. Control cells were treated with the vehicle. Cells were incubated for 3 days. After, cell viability was determined by the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The MTT was added in each well at a final concentration of 0.5 mg mL^{-1} for 4 h at 37 °C. Then medium was aspirated and the formed purple crystals were dissolved with equal volumes of ethanol/DMSO. The optical density (O.D.) was measured using a multiwell plate reader (Multiskan FC, ThermoFisher Scientific, USA) at 540 nm. The percentage of cell viability was calculated from the following equation: Living MDA-MB-231 cells (%) = $O.D._{.test}$ /$O.D._{.control}$ × 100.

Cellular uptake

MDA-MB-231 cells were seeded in glass bottom 8-well tissue culture chambers (SARSTEDT, Germany). Twenty-four hours after, cells were treated with 5 µM concentration of **2**, **4** and **5** for 24 h. After the incubation period, cells were washed three times with culture medium before observation with LSM880 confocal microscope (Carl Zeiss, France). Cells were imaged using a high magnification (63×/1.4 OIL Plan-Apo) at excitation wavelength of 633 nm and emission at 640-703 nm. Phase contrast images were also acquired.

Mannose receptors inhibition

MDA-MB-231 cells were seeded in glass bottom 8-well tissue culture chambers (SARSTEDT, Germany). Twenty-four hours after, cells were treated with 40 mM D-mannose for 1 h, followed by incubation with 5 µM of **5** for 3 h. Control cells were not previously treated with D-mannose and incubated with 5 µM of **5** for 3 h. After the incubation period, cells were imaged as previously described in cellular uptake section.

PDT experiment

MDA-MB-231 cells were seeded in 96-well plates and allowed to grow for 24 h. Then cells were treated with different concentrations of **2**, **4** and **5** for 24 h. Non-treated cells were considered as a control. After incubation, cells were exposed (or not) to light at 650 nm for 1 min, 3 min, 5 min and 10 min. Two days later, the phototoxicity effect was assessed using MTT assay as previously described. For PDT using green light at 545 nm, cells were seeded in 384-well plates and allowed to grow for 24 h. Then cells were treated with different concentrations of **2**, **4** and **5** for 24 h. Non-treated cells were considered as a control. After incubation, cells were exposed (or not) to light at 545 nm for 10 min. The light beam was focused by a microscope objective lens (4×/0.10). Two days later, the phototoxicity effect was assessed using MTT assay as previously described.

Reactive oxygen species (ROS) detection

MDA-MB-231 cells were seeded in 96-well plate. Twenty-four after seeding, cells were treated with 500 nM of **5** for 24 h. After the incubation period, ROS were detected using DCFDA/H2DCFDA-Cellular ROS Assay Kit (Abcam, UK). Control (non-treated) cells and treated cells with **5** were incubated with 20 µM of DCFDA for 45 min at 37 °C, then cells were exposed (or not) to light at 650 nm for 10 min. After irradiation, cells were washed twice then visualized using Leica DM.IRB fluorescence microscope at 450–490 nm.

Statistical analysis

Results were presented as mean ± standard error of the mean (SEM) of three independent experiments. Statistical analysis was performed using GraphPad Prism. The comparison between groups was analyzed with Student's t-test. Differences were considered statistically significant when p values were less than 0.05 (p values < 0.05). The level of significance was defined as *p values <0.05, ** p values < 0.005 and ***p values < 0.0005.

6. References

[1] J-F. Longevial, K. Miyagawa and H. Shinokubo, *Dalton Trans.,* 2020, **49**, 14786.