Electronic supplementary materials *Mendeleev Commun.*, 2026, **36**, 323–325

Photoinactivation of antibiotic-resistant Gram-negative bacteria using charged chlorin photosensitizers

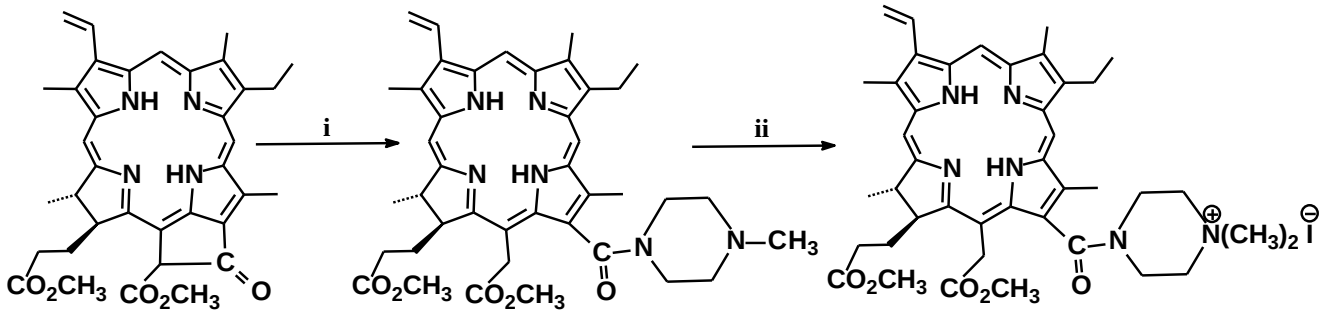
Andrey V. Kustov, Philipp K. Morshnev and Denis S. Lyapin

Contents

S1. Synthesis and identification of 13-(4,4-dimethylpiperazin-4-ium-1-carbonyl)isochlorin *e*$_4$ dimethyl ester iodide (compound 3, MC2)

To obtain monocationic chlorin containing one dialkylpiperazinyl group we performed a simple two-step chemical functionalization of methylpheophorbide *a* **1** shown in Scheme S1. The latter was obtained from *Spirulina platensis* algae *via* alcohol extraction and a simple two-step chemical modification. The opening of the exocycle of methylpheophorbide *a* with *N*-methylpiperazine (Sigma Aldrich, 99%) was performed in the first stage of functionalization. Then, the intermediate (compound **2**) was alkylated with methyl iodide (Sigma Aldrich, >99%) to obtain the chlorin required (compound **3**).

MC2 was identified using ^{1}H and ^{13}C NMR spectra as well as UV–VIS spectroscopy, fluorescence emission spectroscopy and mass spectrometry. ^{1}H and ^{13}C NMR spectra were registered with a Bruker Avance III spectrometer (500 and 125 MHz, respectively). $CDCl_3$ was used as an appropriate solvent with the signal of residual $CHCl_3$ as a standard. Mass spectra were obtained with a MALDI-TOF MS-spectrometer Shimadzu AXIMA Confidence using a 2,5-dihydroxybenzoic acid matrix. UV–VIS and fluorescence spectra were registered with a D8 spectrophotometer (Drawell, China) and a CM 2203 fluorimeter (Solar, Belarus') in highly diluted pigment solutions (~7–10 μmol kg^{-1} for UV–VIS and ~0.8–1 μmol kg^{-1} for fluorescence).



1 **2** **3**

Scheme S1 *Reagents and conditions*: i, *N*-methylpiperazine, $CHCl_3$, 40 °C, 2 h; ii, MeI, $CHCl_3$, room temperature, 1 h.

Methylpheophorbide a (pheophorbide a 17(3)-methyl ester **1**. ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 9.53 (s, 1H, 10-CH), 9.40 (s, 1H, 5-CH), 8.58 (s, 1H, 20-CH), 8.01 [dd, 1H, 3(1)-CH, *J* 17.8 Hz, *J* 6.4 Hz], 6.28 [s, 1H, 13(2)-CH], 6.31 and 6.20 [2d, 1H each, 3(2)-CH_2, *J* 17.9 Hz, *J* 11.6 Hz], 4.48 (q, 1H, 17-CH, *J* 7.5 Hz), 4.23 (d, 1H, 18-CH, *J* 8.5 Hz), 3.90 [s, 3H, 15(3)-Me], 3.69 [q, 2H, 8(1)-CH_2, *J* 7.9 Hz], 3.71 (s, 3H, 17(4)-Me], 3.60 [s, 3H, 12(1)-Me], 3.42 [s, 3H, 2(1)-Me], 3.25 [s, 3H, 7(1)-Me], 2.17–2.69 [4m, 1H each, 17(1)-CH_2, 17(2)-CH_2], 1.83 [d, 3H, 18(1)-Me, *J* 7.4 Hz], 1.71 [t, 3H, 8(2)-Me, *J* 7.6 Hz], 0.57 (br. s, 1H, 23-NH), −1.61 (br. s, 1H, 21-NH). MS (MALDI), *m*/*z* (%): 606.7965 (M^+, 100) (calc. for $C_{36}H_{38}N_4O_5^+$, *m*/*z*: 606.7216), 629.8552 ($M+Na^+$, 49).

13-(4-Methylpiperazin-1-carbonyl)isochlorin e$_4$ *dimethyl ester* **2**. Pheophorbide *a* 17(3)-methyl ester **1** (0.082 mmol) was dissolved in chloroform (2 ml) and *N*-methylpiperazine (3 mmol) was added. The resulting solution was stirred in the dark at 40 °C in an inert atmosphere for 2 h. After repeated washing with water, the organic layer was separated and dried over sodium sulfate. The solution was then filtered and evaporated to dryness, and the final product was thoroughly purified by column chromatography on silica gel using a mixture of chloroform and isopropanol (30 : 1, v/v) as a suitable eluent. The yield of the pigment, obtained as a greenish-purple solid, was 85%. ^{1}H NMR (500 MHz, $CDCl_3$) *δ*: 9.73 [*9.70*] (s, 1H, 10-H), 9.69 [*9.67*] (s, 1H, 5-H), 8.87 [*8.82*] (s, 1H, 20-H), 8.14 [*8.13*] (dd, 1H, *J* 18 Hz, *J* 11.5 Hz, 3-C*H*=CH_2), 6.41 [*6.40*] and 6.38 [*6.37*] [dd, 1H, *J* 18 Hz, *J* 1.5 Hz, 3-CH=CH*H* (*trans*)], 6.19

[*6.18*] and 6.16 [*] [dd, 1H, *J* 11.5 Hz, *J* 1.5 Hz, 3-CH=CHH (*cis*)], 5.81 [*5.46*] and 5.08 [*5.24*] (d each, 2H, *J* 19 Hz, 15-C*H*$_2$CO$_2$Me), 4.49 [*4.48*] (q, 1H, *J* 7.5 Hz, 18-H), 4.34 [*4.42*] (dd, 1H, *J* 9.5 Hz, *J* 2 Hz, 17-H), 3.84 [*] (q, 2H, *J* 8 Hz, 8-C*H*$_2$Me), 3.88 [*3.82*] (s, 3H, 15-CH$_2$CO$_2$Me), 3.67 [*3.64*] (s, 3H, 17-CH$_2$CH$_2$CO$_2$Me), 3.58 [*3.54*] (s, 3H, 12-Me), 3.53 [*3.52*] (s, 3H, 7-Me), 3.36 [*3.35*] (s, 3H, 2-Me); 13-CONC$_4$*H*$_8$NMe$_2^+$I$^-$: 4.70–4.80 [*4.42–4.50*] (br. s, 1H), 3.61–3.73 [*4.02–4.12*] (m, 1H), 2.68–2.75 (m, 3H), 2.85–2.93 (m, 3H), 3.04–3.24 (m, 3H), 2.32–2.38 (m, 3H), 2.44–2.64 (m, 3H), 2.35 [*2.44*] (s, 3H, N-Me); 17-CH$_2$CH$_2$CO$_2$Me: 2.10–2.32 [*] (m, 3H), 1.69–1.75 [*1.86–1.96*] (m, 1H); 1.77 [*1.67*] (d, 3H, *J* 7 Hz, 18-Me), 1.75 [*] (t, 3H, *J* 8 Hz, 8-CH$_2$Me), −1.68 [*−1.57*] (br. s, 1H, 23-NH), −1.88 [*−1.72*] (br. s, 1H, 21-NH). *Note: NMR signals of the minor chlorin isomer are given in italics in brackets and replaced by an asterisk if the value is duplicated.* MS (MALDI), *m*/*z* (%): 707.8270 (M+H$^+$, 100) (calc. for $C_{41}H_{50}N_6O_5^+$, *m*/*z*: 706.8729).

13-(4,4-Dimethylpiperazin-4-ium-1-carbonyl)isochlorin e$_4$ *dimethyl ester iodide* (MC2) **3**. 13-(4-Methylpiperazin-1-carbonyl)isochlorin *e*$_4$ dimethyl ester **2** (0.043 mmol) was dissolved in chloroform (1.5 ml), and methyl iodide (16 mmol) was added. The solution was stirred for 1 h at room temperature. The solvent was then evaporated to dryness, and the final product, obtained in quantitative yield, was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.65 [*9.61*] (s, 1H, 10-H), 9.56 [*9.52*] (s, 1H, 5-H), 8.82 [*8.77*] (s, 1H, 20-H), 8.00 [*] (dd, 1H, *J* 17.5 Hz, *J* 11.5 Hz, 3-C*H*=CH$_2$), 6.32 [*6.30*] [d, 1H, *J* 18 Hz, 3-CH=CH*H* (*trans*)], 6.10 [*] [d, 1H, *J* 11.5 Hz, 3-CH=C*H*H (*cis*)], 5.73 [*5.48*] and 4.95 [*4.94*] (d each, 2H, *J* 18 Hz, 15-C*H*$_2$CO$_2$Me), 4.46 [*] (q, 1H, *J* 7 Hz, 18-H), 4.48 [*4.35*] (br. d, 1H, *J* 9.5 Hz, 17-H), 3.70 [*] (q, 2H, *J* 7 Hz, 8-C*H*$_2$Me), 3.70 [*3.67*] (s, 3H, 15-CH$_2$CO$_2$*Me*), 3.62 [*3.55*] (s, 3H, 17-CH$_2$CH$_2$CO$_2$*Me*), 3.46 [*3.45*] (s, 6H, 7-Me and 12-Me), 3.24 [*3.28*] (s, 3H, 2-Me), 3.02 [*3.08*] and 3.23 [*3.15*] (s each, 6H, N-Me); 13-CONC$_4$*H*$_8$NMe$_2^+$I$^-$: 4.13–4.24 [*4.24–4.33*] (m, 2H), 3.55–3.65 [*3.74–3.94*] (m, 2H), 3.44–3.54 [*] (m, 2H), 3.25–3.37 [*] (m, 1H), 3.12–3.22 [*] (m, 1H); 17-C*H*$_2$C*H*$_2$CO$_2$Me: 2.50–2.60 [*] (m, 1H), 2.10–2.29 [*] (m, 2H), 1.78–1.90 [*] (m, 1H); 1.75 [*1.65*] (d, 3H, *J* 7 Hz, 18-Me), 1.66 [*1.62*] (t, 3H, *J* 8 Hz, 8-CH$_2$*Me*), −1.67 [*−1.58*] (br. s, 1H, 23-NH), −1.52 [*] (br. s, 1H, 21-NH). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 11.28/11.21 [C$^{7(1)}$], 12.15 [C$^{2(1)}$], 13.07/13.30 [C$^{12(1)}$], 17.70 [C$^{8(2)}$], 19.58/19.52 [C$^{8(1)}$], 22.99/22.92 [C$^{18(1)}$], 29.72/29.60 [C$^{17(2)}$], 30.95/31.14 [C$^{17(1)}$], 35.84/35.81 (CH$_2$, piperazinyl fragment), 37.53/37.11 [C$^{15(1)}$], 40.79/41.02 (CH$_2$, piperazinyl fragment), 49.09/49.40 (C^{18}), 51.68/51.32 [C$^{17(4)}$], 52.28/51.70 [C$^{15(3)}$], 52.43/52.32 (N$^+$Me$_2$), 52.59/52.52 (N$^+$Me$_2$), 53.18/53.25 (C^{17}), 60.52 (CH$_2$, piperazinyl fragment), 61.01 (CH$_2$, piperazinyl fragment), 93.95/93.81 (C^{20}), 98.84/98.77 (C^5), 101.82/101.75 (C^{10}), 101.96/102.08 (C^{15}), 122.05/122/10 [C$^{3(2)}$], 122.79/122.98 (C^{13}), 129.17/129.15 [C$^{3(1)}$], 129.35/129.26, 130.74/130.86 (C^{12}, C^2), 134.16/134.04 (C^7), 134.41/134.87 (C^4), 134.92/135.02 (C^3, C^{11}), 136.28/136.21 (C^1), 139.36/139.50 (C^8), 145.31/145.25 (C^{14}), 149.19/149.02 (C^9), 155.07/154.95 (C^6), 167.16/166.76 (C^{16}), 169.39/168.67 (C^{19}), 169.45/169.78 [C$^{13(1)}$], 173.48/173.68 [C$^{17(3)}$], 174.26/173.70 [C$^{15(2)}$]. MS (MALDI), *m*/*z* (%): 722.2762 ([M−I]$^+$, 100) (calc. for $C_{42}H_{53}N_6O_5^+$, *m*/*z*: 721.9074).

According to the NMR data, the final purity of MC2 is > 95%.

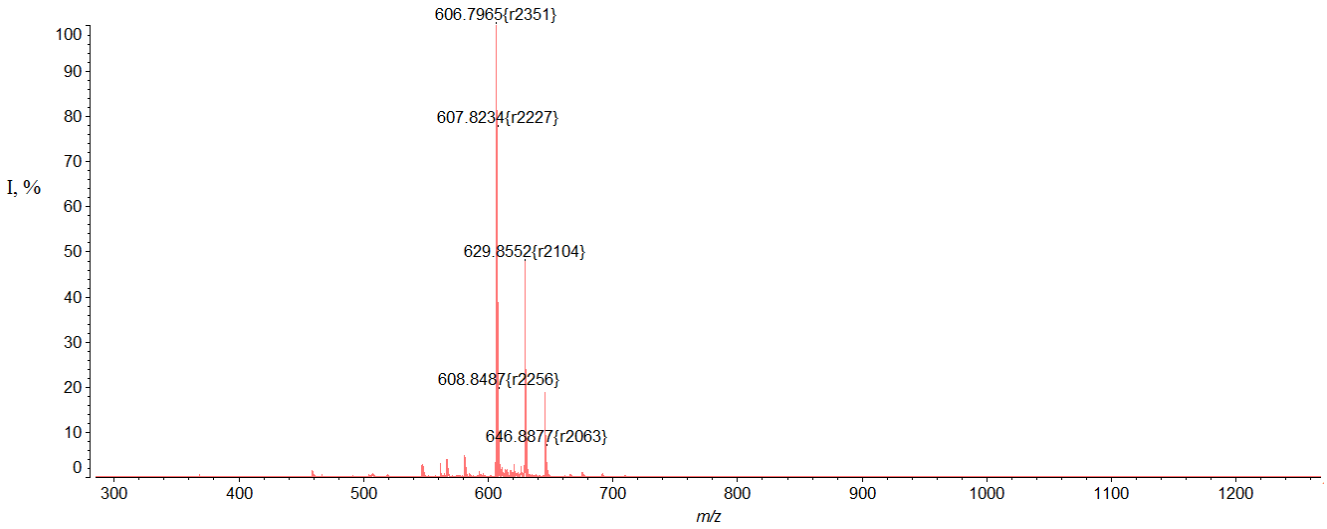


Figure S1 MS (MALDI, DHBA as a matrix) of compound **1**.

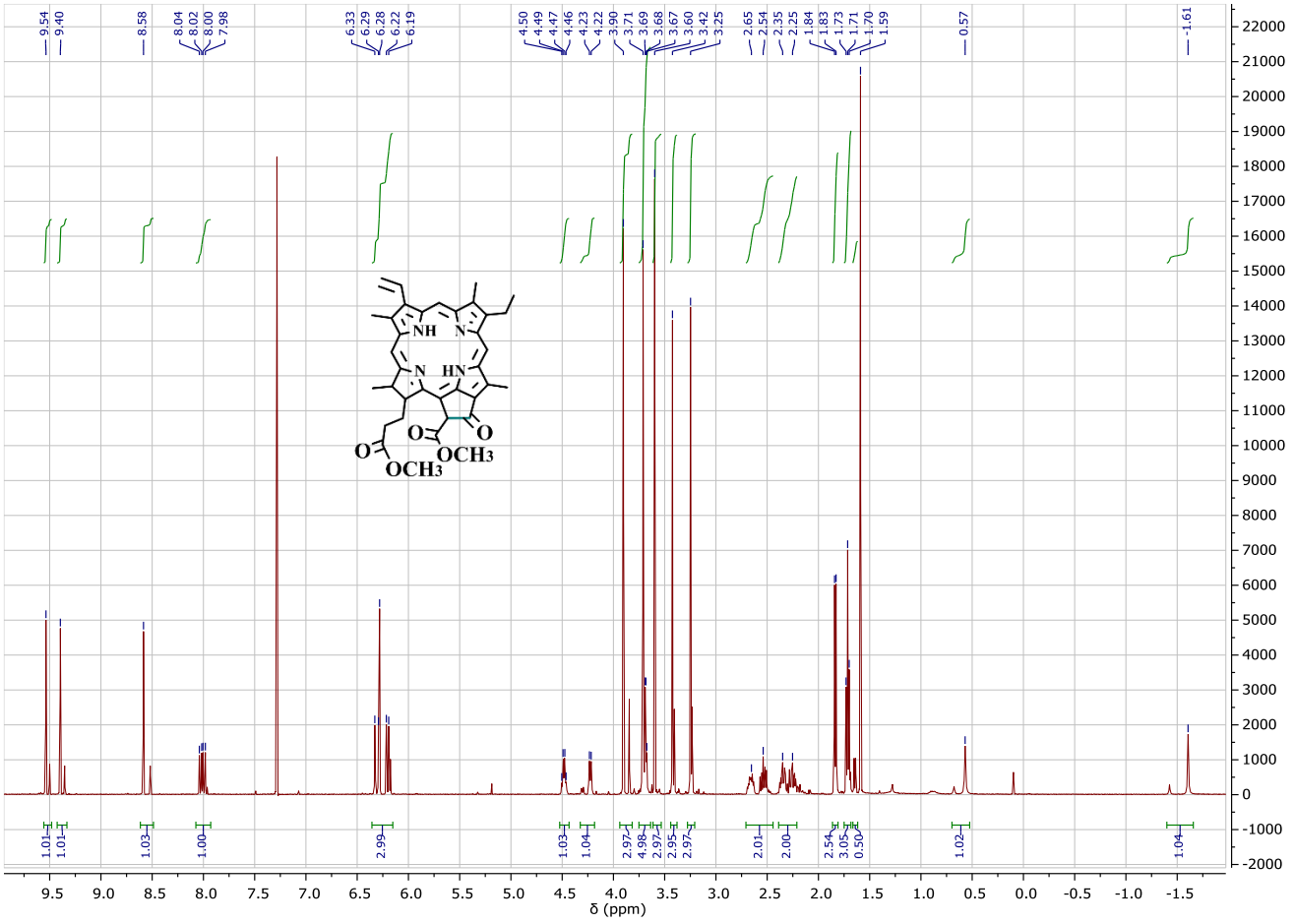


Figure S2 ^{1}H NMR (500 MHz) spectrum of compound **1** in $CDCl_3$.

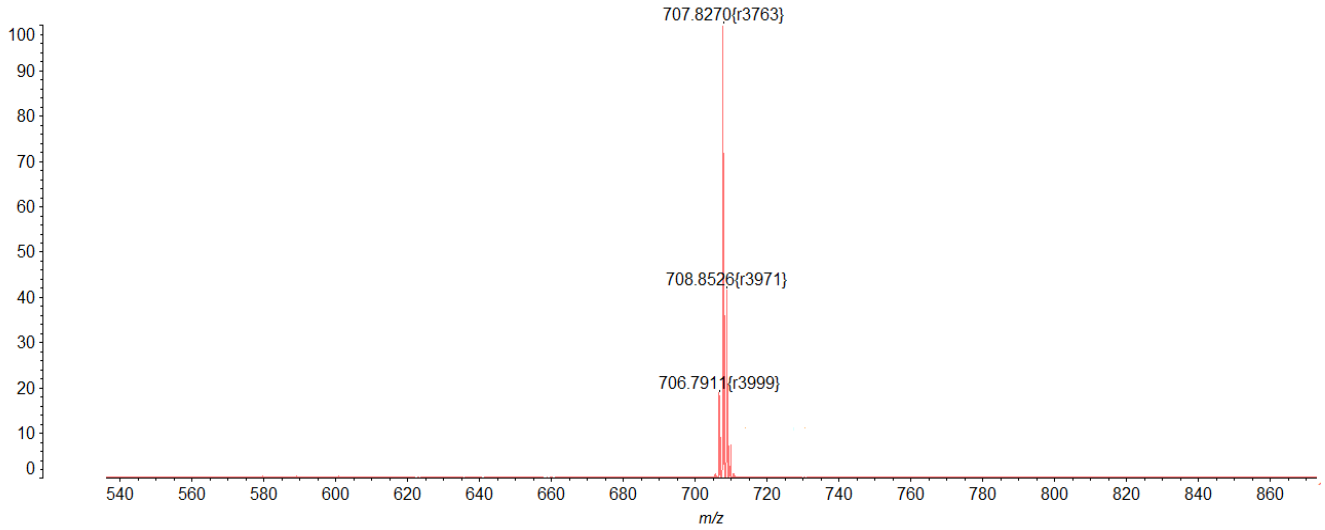


Figure S3 MS (MALDI, DHBA as a matrix) of compound **2**.

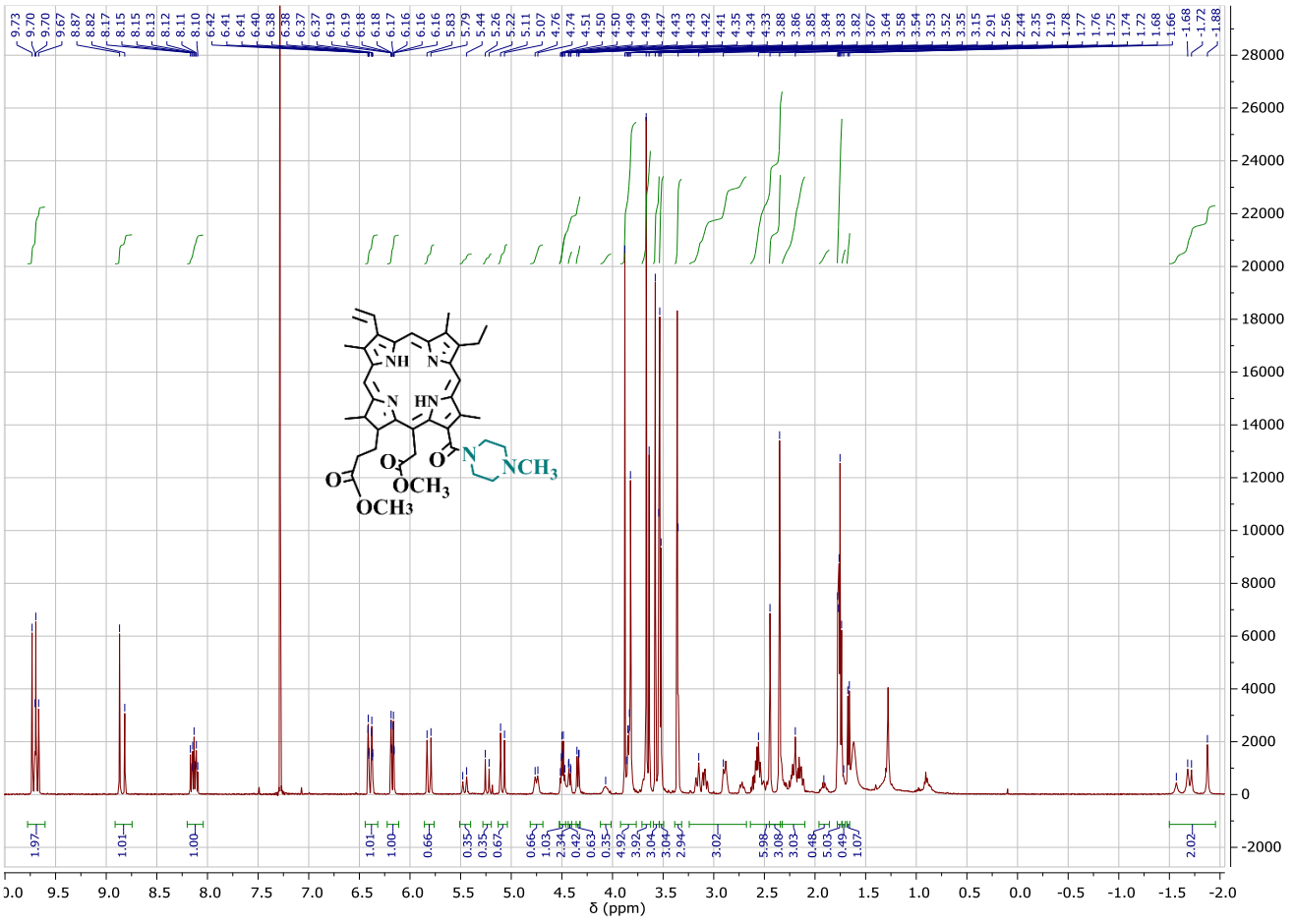


Figure S4 ^{1}H NMR (500 MHz) spectrum of compound **2** in $CDCl_3$.

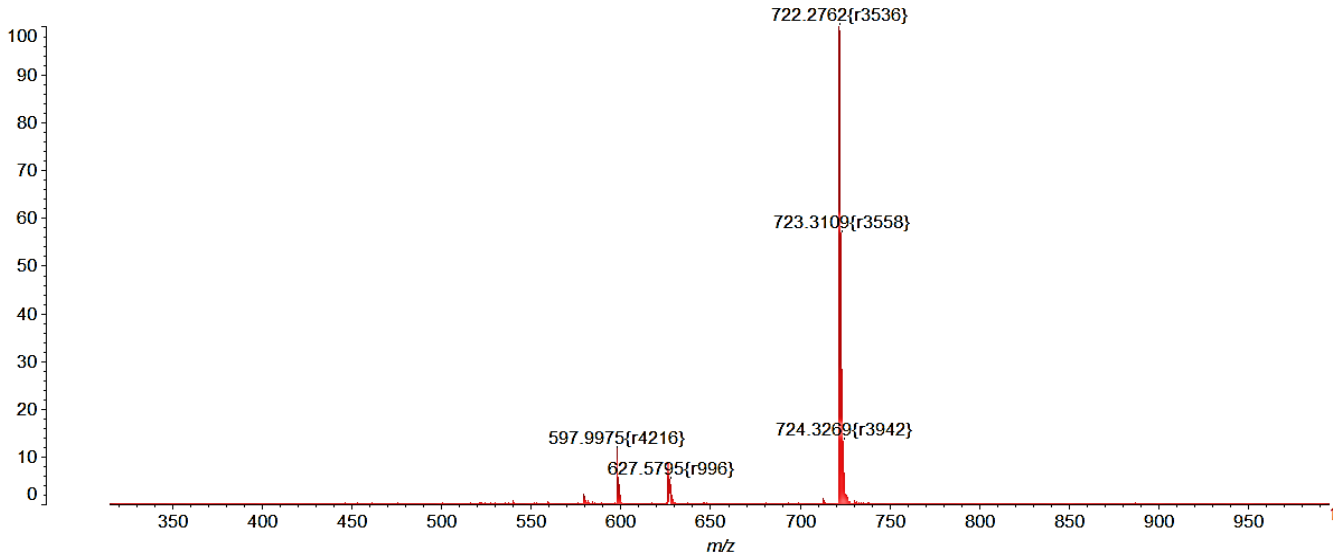


Figure S5 MS (MALDI, DHBA as a matrix) of compound **3.**

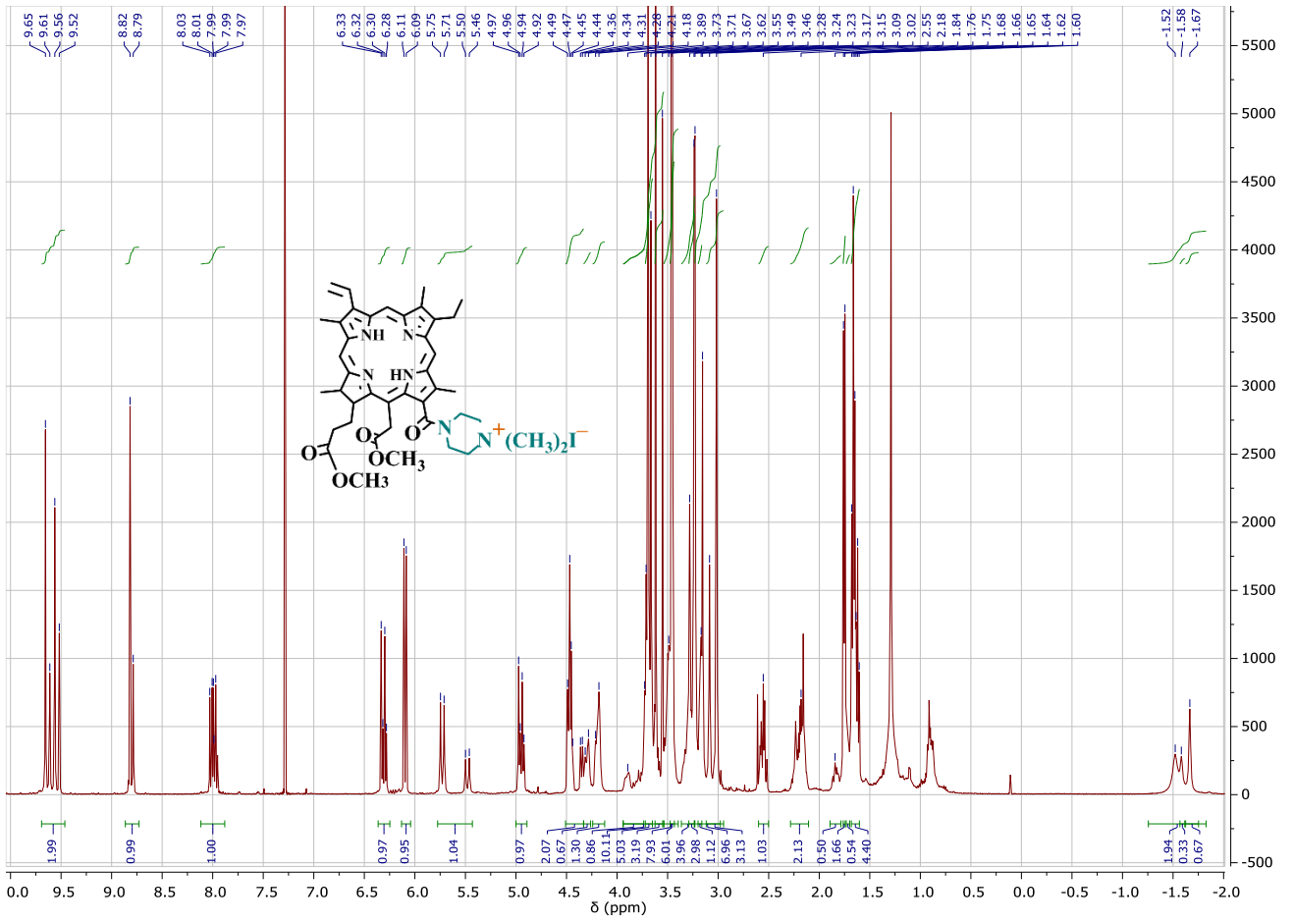


Figure S6 ^{1}H NMR (500 MHz) spectrum of compound **3** in $CDCl_3$.

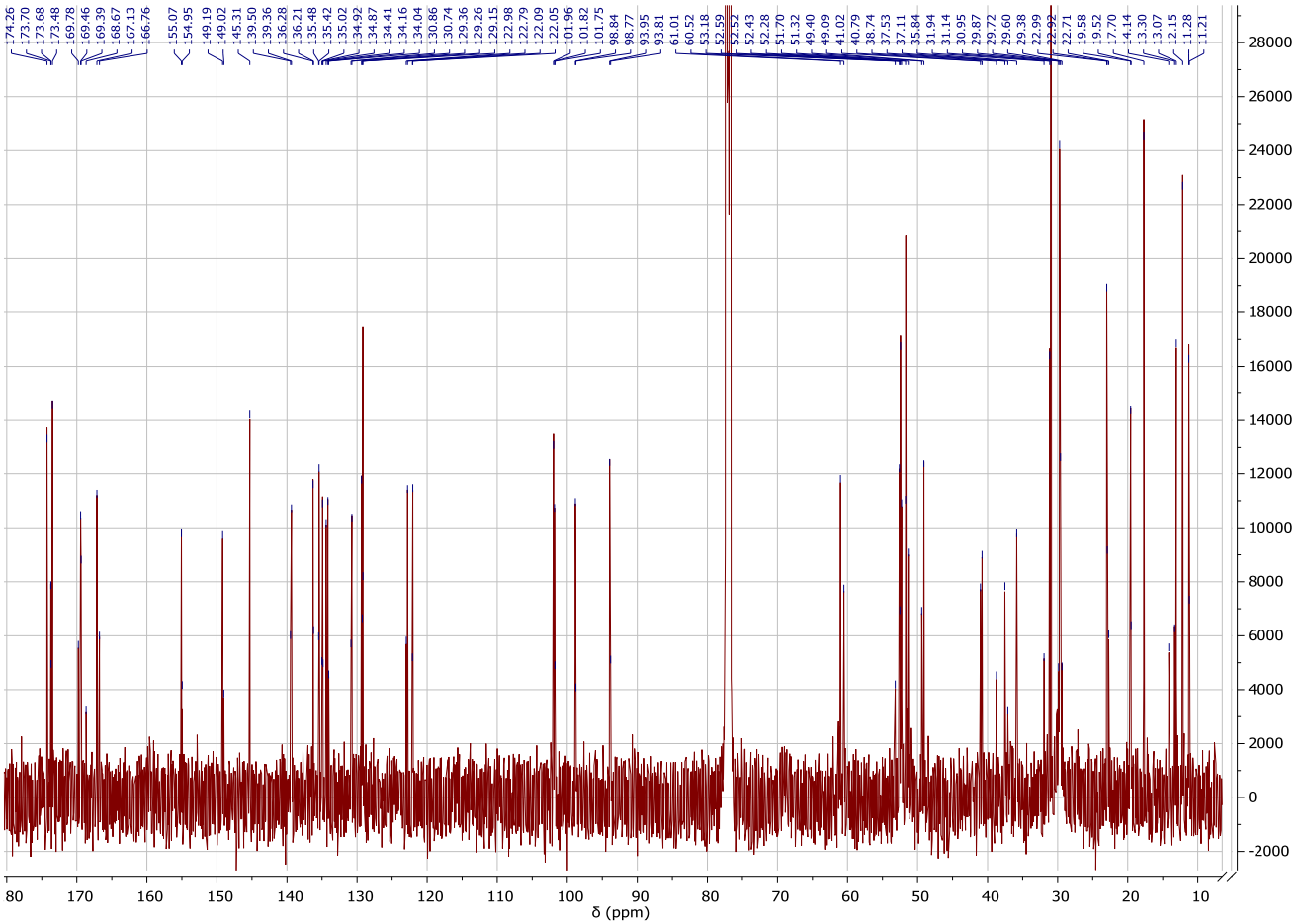


Figure S7 ^{13}C NMR (125 MHz) spectrum of compound **3** in $CDCl_3$.

S2. Chemicals

Methylpheophorbide *a* (Chlorin, Russia) with the initial purity of 95 % was used as supplied. Water was distilled twice in a quartz still to reach the electric conductivity of 1×10^{-5} S m^{-1}. Sodium ethylenediamine tetraacetate ($Na_2H_2EDTA \cdot 2H_2O$), KI and L-tryptophan provided by Panreac (chemical purity) was used as supplied. Chlorin e_6 trisodium salt (chlorin e_6) was purchased from the "RANFARMA" company (Russian Federation) and purified by reprecipitation.

S3. Antimicrobial PDT

S3.1. Preparation of suspension of microbial cells

A nosocomial antibiotic resistant strain of *Pseudomonas aeruginosa* was isolated and accurately grown in the Clinical laboratory of the Ivanovo regional clinical hospital. The pathogen was found to be resistant towards standard doses of "Meropenem", "Cefepime", "Ciprofloxacin" and several other antibiotics but sensitive to "Polymixin B".

The daily cultures of the test-strains were grown using microbiological agar mixed with brain-heart infusion broth or the Ol´kenitskiy medium. The cultures were washed by an appropriate amount of saline and diluted to achieve the concentration of $2.4\text{-}2.7 \cdot 10^8$ colony forming units (CFU) per milliliter (0.8-0.9 according to the McFarland standard). The sowing dose of 10^8 or $2 \cdot 10^7$ CFU ml^{-1} was prepared by a serial dilution of the initial suspension mentioned above.

S3.2. Photoinactivation of Gram (-) bacteria *in vitro*

Half a milliliter of an aqueous PS solution with an appropriate solute concentration was added to each well of the 4-well plate equipped by a lid. Each well contained 0.5 ml of saline with an appropriate bacterial culture (~$2\ 10^7$ CFU·ml^{-1}). After mixing and incubation in the dark during ~ 20 min, the plates were irradiated with a powerful LED panel during 3.5, 7 or 14 min. The total light dose was 20, 40 or 80 J·cm^{-2}. The intensity of the light spot (power density) was measured by an "Argus 03" power meter. To model photoinactivation, the three aliquots of microbial cell suspension were prepared. The first aliquot was an original cell suspension (light control); the second one was a suspension with the PS added which was kept in the dark and the third aliquot was a suspension contained a PS to be irradiated with pre-incubation. The second test-culture was incubated at 37 ^{0}C in the stationary incubator during incubation and irradiation of the first and third aliquots (30-45 min). After all manipulations, the test-cultures were accurately mixed and sown with a sterile calibrated loop on petri dishes containing an appropriate solid growth. After 24-h incubation, the dishes were counted.