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Photoinactivation of the 'ESKAPE' group pathogens by N-confused *meso*-tetrakis(*p*-sulfonatophenyl)porphyrin tetrasodium salt

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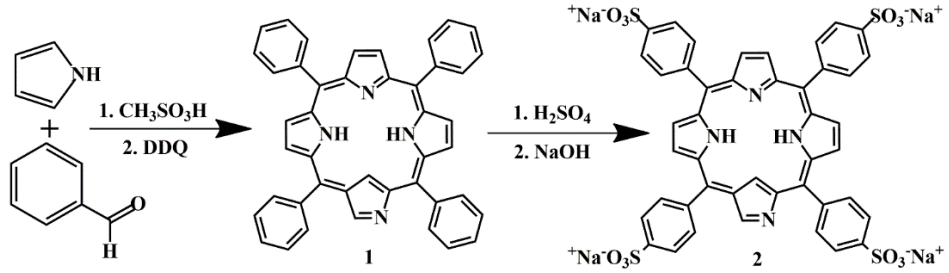
S1. Synthesis and identification

2-Aza-21-carba-meso-tetraphenylporphyrin (NCP, compound **1**) and *2-aza-21-carba-meso-tetrakis(p-sulfonatophenyl)porphyrin tetrasodium salt* (NCPS, compound **2**)

Compound **1** was obtained according to the one-pot Lindsey acid-catalyzed condensation procedure [S1] using reaction of pyrrole and benzaldehyde in dichloromethane in the presence of methanesulfonic acid for 30 minutes and subsequent oxidation of the reaction mixture with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Scheme S1).



Scheme S1 Synthesis of 2-aza-21-carba-*meso*-tetraphenylporphyrin (NCP, comp. *1*) and 2-aza-21-carba-*meso*-tetrakis(*p*-sulfonatophenyl)porphyrin tetrasodium salt (NCPS, comp. **2**).

The product was purified by column chromatography (aluminium oxide (activity grade III), petroleum ether: dichloromethane) to give a violet solid, yield: 23 %. The spectral data of comp. **1** are presented in Figures S1-S3.

^{1}H NMR spectra were registered with a Bruker Avance III spectrometer (500 MHz, Germany). $CDCl_3$ and D_2O were used as an appropriate solvents and searches of an internal standard. MS-spectra were obtained with a MALDI FAB MS spectrometer (Shimadzu AXIMA Confidence, Japan) using o-dihydroxybenzoic acid (DHB) as a matrix. UV–Vis were registered at room temperature with a CM 2203 spectrofluorimeter (Solar, Belarus') in highly diluted porphyrinoid solutions.

Compound **1.** ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 9.00 and 8.94 (2d, 1H each, 7-H and 18-H, J = 4.5 Hz), 8.77 (s, 1H, 3-H), 8.55, 8.56, 8.58 and 8.62 (4d, 1H each, 8,12,13,17-H, J = 4.5 Hz), 8.38 and 8.34 (2d, 2H each, 5- and 20- *o*-Ph-H, J = 7.5 Hz), 8.17 (m, 4H, 10- and 15- *o*-Ph-H), 7.80−7.90 (m, 6H, *m*- and *p*- Ph-H), 7.75−7.80 (m, 6H, *m*- and *p*- Ph-H), -2.35 (s, 2H, 22- and 24- NH), -4.90 (s, 1H, 21-CH). MS (MALDI-TOF): *m/z* (%): 615.3196 (100) $[M+1]^+$, 1227.5524

(15%) $[2M-H]^+$. calcd. for $C_{44}H_{30}N_4$, *m/z* 614.7358. UV ($CHCl_3$, λ/nm (lgε)): 437 (Soret, 5.38), 505 (3.95), 541 (4.32), 684 (.44), 729 (4.41); UV (DMF, λ/nm (lgε)): 442 (Soret, 5.35), 545 (3.96), 594 (4.08), 644 (4.24), 698 (4.34).

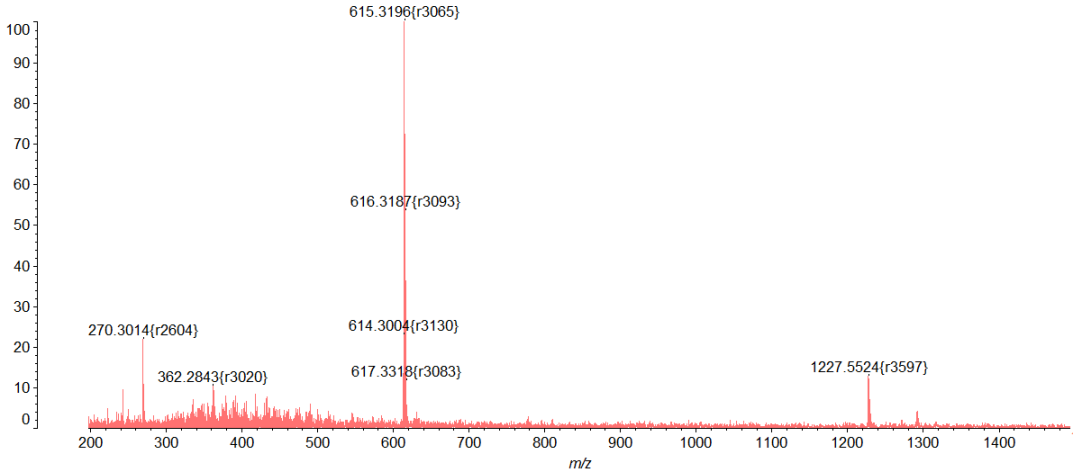


Figure S1. Mass spectrum (MALDI-TOF) of 2-aza-21-carba-*meso*-tetraphenylporphyrin (NCP, comp. **1**). DHB was used as a matrix.

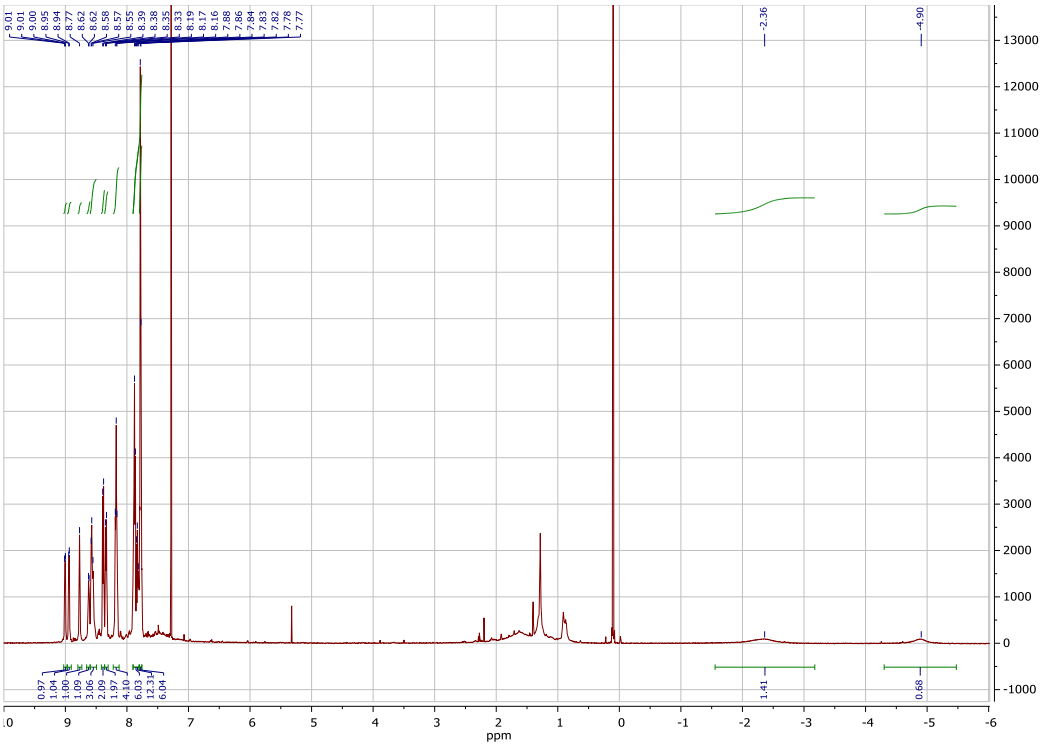


Figure S2. ^{1}H NMR spectrum of 2-aza-21-carba-*meso*-tetraphenylporphyrin (NCP, comp. **1**) in $CDCl_3$. The sample contains solvent signals (CH_2Cl_2, H_2O) as well as signals of TMS and solvent impurities. The chemical shift values are indicated in ppm.

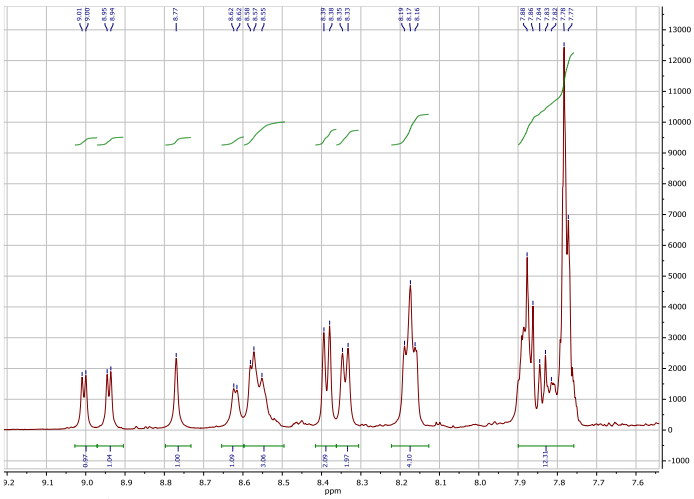


Figure S3. Expansion of ^{1}H NMR spectrum of 2-aza-21-carba-*meso*-tetraphenylporphyrin (NCP, comp. **1**) in $CDCl_3$ (7.5-9.2 ppm).

For sulfonation, NCP was heated in concentrated sulfuric acid for 5 hours. The excess reagent was neutralised with sodium hydroxide (see Scheme S1) [S2]. The final product was purified by, firstly, extraction with methanol using a Soxhlet apparatus and, secondly, by column chromatography (Bio-Beads S-X1 Support, eluent – methanol). A green solid was obtained in 37% yield. The spectral data of comp. **2** are presented in Fig. S4. The product obtained was insoluble in $CDCl_3$ and other low polar solvents. The signals of the NMR spectrum determined in D_2O were not recognizable due to strong solute aggregation and tautomeric equilibria [S3].

UV (EtOH, λ/nm): 438 (Soret), 539, 581, 654,740; UV (DMF, λ/nm): 444 (Soret), 548, 596, 646, 701; UV (H_2O, λ/nm): 373,452 (Soret), 571, 647, 775.

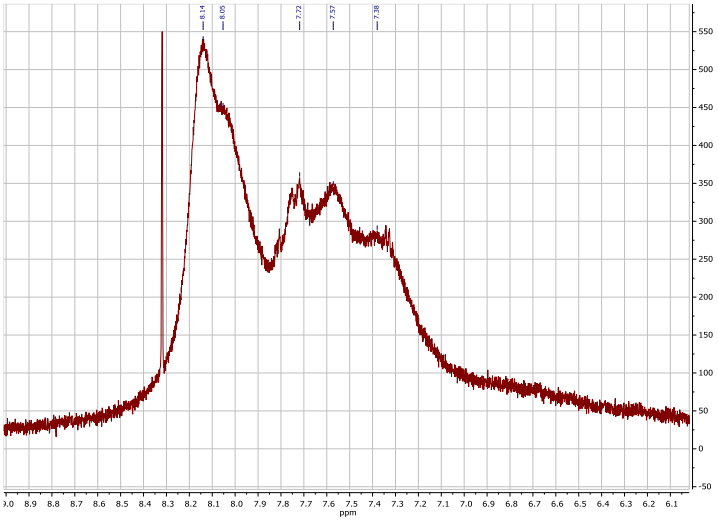


Figure S4. ^{1}H NMR spectrum of 2-aza-21-carba-tetrakis(*p*-sulfonatophenyl)porphyrin tetrasodium salt (comp. **2**) in D_2O. The chemical shift values are indicated in ppm.

S2. PS aggregation in aqueous solutions

Dynamic light scattering (DLS) of aqueous solutions of comp. **2** was studied with a Zetasizer Nano ZS apparatus ZEN 3600 (Malvern Instruments, Great Britain) equipped by a laser with λ = 633 nm and a non-invasive backscatter technology. The results are shown in Fig. S5.

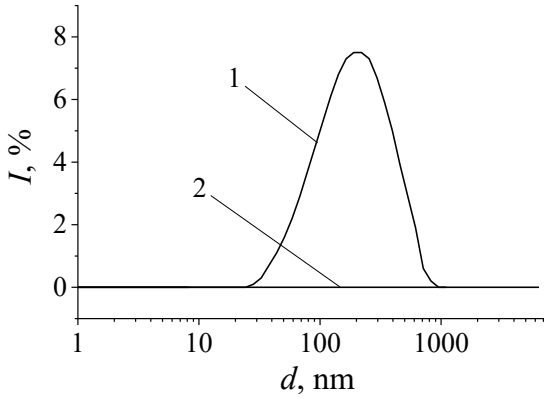


Figure S5. Dynamic light scattering in an aqueous solution (m_{PS} ~ 10^{-6} mol kg^{-1}) of comp. **2** without (1) and with (2) an equimolar amount of Tween 80. The PS solutions were stored in glass vessels protected from sunlight. The experiments were performed a couple of days after the preparation of solutions, and the intensity distribution of scattering was analyzed.

Nanoaggregates formed in micromolal aqueous solutions of comp. **2** totally disappear in the presence of non-ionic surfactant Tween 80 taken in the molar ratio of 1:1 (Fig. S5).

S3. The interaction of PSs with potential carriers

The example of titration of comp. **1** by Tween 80 is given in Table S1.

Table S1. Spectrophotometric titration of an aqueous solution of comp. 1 (m_{PS}=·7.3 10^{-6} mol·kg^{-1}) containing 10% of EtOH by Tween 80

Comp. **1**, λ= 710 nm		Comp. **1**, λ= 710 nm	
$m_{Tween\ 80}\cdot 10^4$, mmol kg^{-1}	*A*, r.u.	$m_{Tween\ 80}\cdot 10^4$, mmol kg^{-1}	*A*, r.u.
0	0.196	0.146	0.513
0.0073	0.297	0.219	0.523
0.0219	0.436	0.292	0.525
0.0365	0.462	0.365	0.528
0.0511	0.481	0.511	0.530
0.073	0.508	0.584	0.531
		0.657	0.531

The experimental *A*- *f* ($m_{Tween\ 80}$) curves were fitted to the model equation to obtain the lg K_b and *n* parameters of PS-Tween 80 binding (Fig. S6):

$$\log[(A - A_0)/(1 - (A - A_0))] = \lg(K_b) + n\times\lg[m_{Tm} - n\times m_{PS}\cdot(A - A_0)/(A_{max} - A_0)] \quad (S1),$$

where m_{PS} is the PS molality of 7 10^{-6} mol·kg^{-1}; $m_{Tm} = m_T$ − CMC is the molality of aggregated Tween 80 which is evaluated as the difference between its analytical concentration and the critical micellar concentration, *i.e.* CMC=1.2 10^{-5} M; *n* is the mean number of Tween 80

molecules in contact with a PS in a micelle; A_0 and A_{max} are the optical densities of free (A_0) and totally bound to a micelle (A_{max}) PS. The results obtained are given in Table S2 for both PSs.

Table S2. Binding parameters of comps. **1**, **2** to Tween 80 micelles

PS	m_T mol kg^{-1}	logK_b	n	R^2
Comp. **1**	$(0.07\text{-}5.1)\cdot10^{-4}$	2.76 ± 0.11	0.56	0.985
Comp. **2**	$(0.37\text{-}14.6)\cdot10^{-4}$	2.28 ± 0.16	0.58	0.942

S3. Antimicrobial PDT

S3.1. Preparation of suspension of microbial cells

Archival or nosocomial antibiotic resistant strains, *viz*. *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter baumannii* were isolated and accurately grown in the Clinical laboratory of the Ivanovo regional clinical hospital. *Pseudomonas aeruginosa* was found to be resistant towards standard doses of "Meropenem", "Cefepime", "Ciprofloxacin", *etc*., but sensitive to "Polymixin B". *Acinetobacter baumannii* is resistant towards "Meropenem", "Imipenem", "Ciprofloxacin", *etc*., 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 with an appropriate amount of saline and diluted to achieve the concentration of $2.4\text{-}2.7\cdot10^8$ colony forming units (CFU) per milliliter (0.8-0.9 according to the McFarland standard). The sowing dose of 10^8 or $2\cdot10^7$ 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 20-25 min, the plates were irradiated with a powerful LED panel during 7, 14 or 28 min. The total light dose was 40, 80 or 160 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 the PS to be irradiated with pre-incubation. The second test-culture was incubated at 37 °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 mixed

and sown with a sterile calibrated loop on petri dishes containing an appropriate solid growth. After 24-h incubation, the dishes were counted.

References:

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S2. A. P. Thomas, P. S. S. Babu, S. A. Nair, S. Ramakrishnan, D. Ramaiah, T. K. Chandrashekar, A. Srinivasan and M. R. Pillai, *J. Med. Chem.*, 2012, **55**, 5110; https://doi.org/10.1021/jm300009q.

S3. T. Ishizuka, R. Sakashita, O. Iwanaga, T. Morimoto, S. Mori, M. Ishida, M. Toganoh, K. Takegoshi, A. Osuka and H. Furuta, *J. Phys. Chem. A*, 2020, **124**, 5756; https://doi.org/10.1021/acs.jpca.0c04779.