Mendeleev Communications

Mendeleev Commun., 2025, **35**, 611–613

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

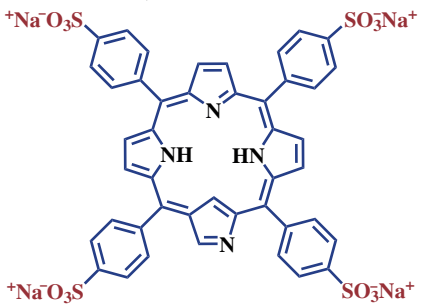
Andrey V. Kustov,*[a] Philipp K. Morshnev[a] and Dmitry B. Berezin[b]

[a] *G. A. Krestov Institute of Solution Chemistry, Russian Academy of Sciences, 153045 Ivanovo, Russian Federation. E-mail: kustov26@yandex.ru*

[b] *Institute of Macroheterocyclic Compounds, Ivanovo State University of Chemistry and Technology, 153012 Ivanovo, Russian Federation*

DOI: 10.71267/mencom.7734

A polyanionic water-soluble N-confused porphyrin photosensitizer containing four *p*-sulfonatophenyl groups was synthesized, and its behavior was studied in water and aqueous solutions of the micellar surfactant Tween 80. The antimicrobial photodynamic inactivation study indicated that this photosensitizer efficiently killed *Staphylococcus aureus*; however, Gram-negative pathogens having an outer lipopolysaccharide membrane were relatively resistant to this compound. The potentiation of photodynamic inactivation by addition of appropriate biocompatible agents increasing permeability of the membrane above was successfully tested and briefly discussed.



Keywords: antibiotic resistance, Gram-positive and Gram-negative bacteria, photodynamic inactivation, photosensitizers, potentiating agents, porphyrins.

Pathogenic microorganisms can develop resistance against a variety of costly antimicrobial agents including last generation antibiotics.[1–3] This problem largely derives from their single mode of action, and it is a matter of time for bacteria to become insensitive towards a recently efficient drug.[4–6] Special attention is paid to the so-called 'ESKAPE' group[3–5,7,8] containing both Gram-positive and Gram-negative pathogenic superbugs, *viz*., *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp., which in the future will be capable of resisting all known antibiotic agents.

Antimicrobial photodynamic therapy (aPDT) is a promising technology of treating many localized microbial infections.[8–11] This approach affecting several targets in microbial cells offers several advantages over a standard antibiotic treatment but still remains underutilized in clinical practice.[11–14] The crucial component of aPDT is a light-sensitive photosensitizing molecule that can rapidly bind to or penetrate into a bacterial wall and generate singlet oxygen ($^1O_2$) and/or other toxic reactive oxygen species ($HO^•$, $O_2^{•-}$) under appropriate irradiation. In recent years, the confused porphyrins as potential photosensitizers (PSs) attract special attention due to their near-IR absorption and emission as well as established synthetic protocols for their preparation.[15–17] N-Confused porphyrin **1**, 2-aza-21-carba-*meso*-tetraphenylporphyrin, seems to be a promising platform for the derivatization to access the PSs with the required properties.

Here, we describe the results of photodynamic inactivation of several microorganisms belonging to the 'ESKAPE' group with the water-soluble inverted porphyrin-type PS, *viz*. N-confused 2-aza-21-carba-*meso*-tetrakis(*p*-sulfonatophenyl)porphyrin tetrasodium salt **2**. The brief description of synthesis and identification of compounds **1** and **2** is given in Online Supplementary Materials, the results of bacteria photoinactivation *in vitro* are compiled in Table 1.

Figure 1 illustrates the absorption spectra of compound **2** in water, ethanol and DMF. The absorption spectra contain the intensive Soret (*B*-) band in the blue light region at λ = 435–455 nm and much less pronounced *Q*-bands in the green and red light regions between 500 and 800 nm. The difference in the UV–VIS spectra in protic EtOH and aprotic DMF can be explained by the solvent-dependent tautomerism specific for all

Table 1 Photoinactivation of archival and antibiotic resistant pathogens *in vitro* with porphyrin **2**.

Photosensitizer **2** loading/μmol kg^{-1} (additive)	Colony forming units (CFU) at light dose/ J cm^{-2}			
	none (dark)	40	80	160
Archival *Staphylococcus aureus*				
100 (+1% Tween 80)	10^7	15	0	0
Archival *Escherichia coli*				
100 (+1% Tween 80)	8×10^6	10^7	7×10^6	3×10^6
Nosocomial *Pseudomonas aeruginosa*				
50	7×10^6	–	3×10^6	7×10^5
50 (+0.05% EDTA)[a]	2×10^6	–	10^5	0
50 (+0.025% ε-Pl)[a]	5×10^5	–	4×10^3	90
Nosocomial *Acinetobacter baumannii*				
50	3×10^6	–	7×10^5	4×10^5
50 (+0.05% EDTA)[a]	1×10^6	–	0	0
50 (+0.025% ε-Pl)[a]	6×10^5	–	1×10^3	7×10^2

[a]EDTA denotes disodium ethylenediaminetetraacetate, and ε-Pl is ε-polylysine (N = 30).

© 2025 Mendeleev Communications

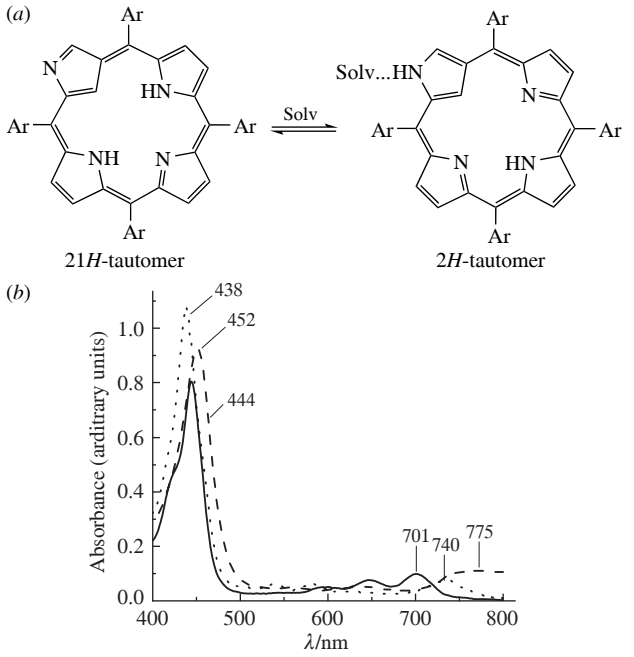


Figure 1 (*a*) Solvent-dependent tautomers of 2-aza-21-carba-*meso*-tetrakis(*p*-sulfonatophenyl)porphyrin tetrasodium salt (compound **2**) and (*b*) its absorption spectra (C_{PS} ~ 1×10^5 mol dm^{-3}) in DMF (solid line, 2*H*-tautomer), EtOH (dotted line, 21*H*-tautomer) and water (dashed line, aggregated form of **2**).

N-confused porphyrins.[15,18] The solute transfer from EtOH to DMF shifts the tautomeric equilibrium above from 21*H* to 2*H* species, while the broadening and red shift of the absorption bands in water result from a strong hydrophobic association of 21*H*-form of compound **2**. The dynamic light scattering study supports this finding (see Online Supplementary Materials), indicating the existence of significant concentration fluctuations in aqueous solutions of even at μM PS concentrations.

Recently,[19–23] we have shown that many water-soluble porphyrin, chlorin and phthalocyanine PSs formed stable complexes with the biocompatible non-ionic surfactant Tween 80,[24] the titration curve demonstrating two modes of binding. The two hundredfold molar excess of the surfactant usually leads to total PS-Tween 80 binding and prevents PS–PS contacts in a micellar phase.[21] However, both confused porphyrins **1** and **2** form much less stable complexes with the surfactant, and the logK_b values obtained are of 2 (see Online Supplementary Materials). Hence, Tween 80 seems to be not an appropriate carrier for this type of light-sensitive molecules.

Table 1 compares dark and light induced toxicity towards several archival and nosocomial antibiotic resistant bacterial pathogens. The sowing dose was 10^7 CFU, and the light control without PS gives 7×10^6, 6×10^6, 8×10^6 and 5×10^6 CFU values for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, respectively. Inspection of the data compiled in Table 1 shows that compound **2** provides seven logs of killing (total elimination) towards *Staphylococcus aureus* even at a light dose of 40 J cm^{-2}, while Gram-negative pathogen *Escherichia coli* remains totally resistant even at a light dose of 160 J cm^{-2}. Similar resistance reveals the nosocomial strains. This finding is in total agreement with our previous studies using neutral and anionic macrocyclic photosensitizers.[9–11,23,25,26] The results obtained clearly indicate that the polyanionic porphyrin is not able to penetrate into or bind to the outer membrane of Gram-negative bacteria. It is apparent that the similar situation was observed for octaanionic phthalocyanines[23] and *meso*-tetrakis(*p*-sulfonatophenyl)-porphyrin.[27,28] The interaction of Tween 80 micelles with the PS seems to prevent at least in part hydrophobic aggregation of macrocycles. However, singlet oxygen species generated far from appropriate targets in microbial cells are not able to cause fatal damage towards pathogenic microorganisms. Thus, the enhancement in photodynamic activity of compound **2** can be achieved with potentiating agents providing an increase in permeability of the lipopolysaccharide outer membrane mentioned above.[9,29–32] We see that both PS formulations being almost non-toxic in the dark, significantly enhance photoinactivation of both nosocomial antibiotic resistant pathogens. It is worth noting that the PS formulation containing EDTA is less toxic in the dark compared to the formulation with polylysine but provides more efficient killing under irradiation.

In summary, our results indicate that polyanionic confused porphyrins have some potential in the antimicrobial photodynamic therapy. However, the efficient bacterial killing with this type of light-sensitive molecules needs both potentiating agents and larger light doses compared to the chlorin-type photosensitizing agents.[31,32]

This research was funded by the Russian Science Foundation (grant no. 25-23-00037; https://rscf.ru/project/25-23-00037, accessed on December 28, 2024).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7734.

References

1 W. R. Miller, A. S. Bayer and C. A. Arias, *Cold Spring Harb. Perspect. Med.*, 2016, **6**, a026997; https://doi.org/10.1101/cshperspect.a026997.
2 M. Hamidian and S. J. Nigro, *Microb. Genom.*, 2019, **5**, e000306; https://doi.org/10.1099/mgen.0.000306.
3 W. R. Miller and C. A. Arias, *Nat. Rev. Microbiol.*, 2024, **22**, 598; https://doi.org/10.1038/s41579-024-01054-w.
4 T. Hwang, K. A. Gibbs, S. H. Podolsky and J. A. Linder, *Lancet Infect. Dis.*, 2015, **15**, 1000; https://doi.org/10.1016/S1473-3099(15)00235-2.
5 B. Spellberg, J. G. Bartlett and D. N. Gilbert, *N. Engl. J. Med.*, 2013, **368**, 299; https://doi.org/10.1056/NEJMp1215093.
6 Yu. S. Bortnevskaya, V. A. Malikova, N. Yu. Karpechenko, N. A. Bragina and K. A. Zhdanova, *Mendeleev Commun.*, 2024, **34**, 685; https://doi.org/10.1016/j.mencom.2024.09.019.
7 D. M. P. De Oliveira, B. M. Forde, T. J. Kidd, P. N. A. Harris, M. A. Schembri, S. A. Beatson, D. L. Paterson and M. J. Walker, *Clin. Microbiol. Rev.*, 2020, **33**, e00181; https://doi.org/10.1128/cmr.00181-19.
8 Y. Liu, R. Qin, S. A. J. Zaat, E. Breukink and M. Heger, *J. Clin. Transl. Res.*, 2015, **1**, 140; https://dx.doi.org/10.18053/jctres.201503.002.
9 M. R. Hamblin, *Curr. Opin. Microbiol.*, 2016, **33**, 67; https://doi.org/10.1016/j.mib.2016.06.008.
10 M. R. Hamblin, *Expert Rev. Anti.-Infect. Ther.*, 2017, **15**, 1059; https://doi.org/10.1080/14787210.2017.1397512.
11 A. V. Kustov, D. B. Berezin, A. I. Strelnikov and N. P. Lapochkina, *Protivoopukholevaya i antimikrobnaya fotodinamicheskaya terapiya: mekhanizmy, misheni, kliniko-laboratornye issledovaniya* (*Antitumor and Antimicrobial Photodynamic Therapy: Mechanisms, Targets, Clinical and Laboratory Studies: A Guide*), ed. A. K. Gagua, Largo, Moscow, 2020 (in Russian); https://elibrary.ru/download/elibrary_44327224_90871407.pdf.
12 M. Wainwright, T. Maisch, S. Nonell, K. Plaetzer, A. Almeida, G. P. Tegos and M. R. Hamblin, *Lancet Infect. Dis.*, 2017, **17**, e49; https://doi.org/10.1016/S1473-3099(16)30268-7.
13 A. V. Kustov, D. B. Berezin, V. P. Zorin, P. K. Morshnev, N. V. Kukushkina, M. A. Krestyaninov, T. V. Kustova, A. I. Strelnikov, E. V. Lyalyakina, T. E. Zorina, O. B. Abramova and E. A. Kozlovtseva, *Pharmaceutics*, 2023, **15**, 61; https://doi.org/10.3390/pharmaceutics15010061.
14 J. Huang, Q. Fan, L. Shi, J. Shen and H. Wang, *Photodiagn. Photodyn. Ther.*, 2024, **48**, 104300; https://doi.org/10.1016/j.pdpdt.2024.104300.
15 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.

16 M. Toganoh and H. Furuta, *Chem. Rev.*, 2022, **122**, 8313; https://doi.org/10.1021/acs.chemrev.1c00065.
17 S. V. Kurmaz, I. V. Ulyanov, N. S. Emelyanova, V. A. Kurmaz, A. V. Kozlov, L. R. Sizov and A. Yu. Rybkin, *Mendeleev Commun.*, 2024, **34**, 492; https://doi.org/10.1016/j.mencom.2024.06.007.
18 D. B. Berezin and D. R. Karimov, *Macroheterocycles*, 2009, **2**, 42; https://doi.org/10.6060/mhc2009.1.42.
19 D. B. Berezin, S. O. Kruchin, N. V. Kukushkina, E. A. Venediktov, M. O. Koifman and A. V. Kustov, *Photochem.*, 2023, **3**, 171; https://doi.org/10.3390/photochem3010011.
20 A. V. Kustov, M. A. Krestyaninov, S. O. Kruchin, O. V. Shukhto, T. V. Kustova, D. V. Belykh, I. S. Khudyaeva, M. O. Koifman, P. B. Razgovorov and D. B. Berezin, *Mendeleev Commun.*, 2021, **31**, 65; https://doi.org/10.1016/j.mencom.2021.01.019.
21 A. V. Kustov, Ph. K. Morshnev, O. V. Shukhto, N. L. Smirnova, N. V. Kukushkina, O. I. Koifman and D. B. Berezin, *Russ. Chem. Bull.*, 2023, **72**, 566; https://doi.org/10.1007/s11172-023-3820-2.
22 D. B. Berezin, V. V. Makarov, V. V. Aleksandriyskiy, M. A. Krestyaninov, T. V. Kustova, P. B. Razgovorov and A. V. Kustov, *J. Appl. Spectrosc.*, 2023, **90**, 526; https://doi.org/10.1007/s10812-023-01563-1.
23 D. B. Berezin, V. V. Makarov, S. A. Znoyko, V. E. Mayzlish and A. V. Kustov, *Mendeleev Commun.*, 2020, **30**, 621; https://doi.org/10.1016/j.mencom.2020.09.023.
24 L. S. Schwartzberg and R. M. Navari, *Adv. Ther.*, 2018, **35**, 754; https://doi.org/10.1007/s12325-018-0707-z.
25 L. Huang, T. Dai and M. R. Hamblin, in *Photodynamic Therapy: Methods and Protocols*, ed. C. J. Gomer (*Methods in Molecular Biology*, Springer, vol. 635), Humana Press, 2010, pp. 155–173; https://doi.org/10.1007/978-1-60761-697-9_12.
26 N. Suvorov, V. Pogorilyy, E. Diachkova, Y. Vasil'ev, A. Mironov and M. Grin, *Int. J. Mol. Sci.*, 2021, **22**, 6392; https://doi.org/10.3390/ijms22126392.
27 A. Hanakova, K. Bogdanova, K. Tomankova, K. Pizova, J. Malohlava, S. Binder, R. Bajgar, K. Langova, M. Kolar, J. Mosinger and H. Kolarova, *Microbiol. Res.*, 2014, **169**, 163; https://doi.org/10.1016/j.micres.2013.07.005.
28 R.-M. Ion, *Coatings*, 2021, **11**, 393; https://doi.org/10.3390/coatings11040393.
29 C. Vieira, A. T. P. C. Gomes, M. Q. Mesquita, N. M. M. Moura, M. G. P. M. S. Neves, M. A. F. Faustino and A. Almeida, *Front. Microbiol.*, 2018, **9**, 2665; https://doi.org/10.3389/fmicb.2018.02665.
30 N. S. Gsponer, M. B. Spesia and E. N. Durantini, *Photodiagn. Photodyn. Ther.*, 2015, **12**, 67; https://doi.org/10.1016/j.pdpdt.2014.12.004.
31 A. V. Kustov, *ChemChemTech*, 2023, **66**, 32; https://doi.org/10.6060/ivkkt.20236612.6902.
32 A. V. Kustov, N. V. Kukushkina, E. V. Lyalyakina, N. N. Solomonova, A. K. Gagua, O. I. Koifman and D. B. Berezin, *Dokl. Chem.*, 2023, **508**, 32; https://doi.org/10.1134/S0012500823700155.

Received: 28th January 2025; Com. 25/7734