Available online at www.sciencedirect.com

ScienceDirect

*Mendeleev Commun.*, 2020, **30**, 159–161

Mendeleev Communications

# Synthesis and antiproliferative activity of new chlorin $e_6$ glycoconjugates

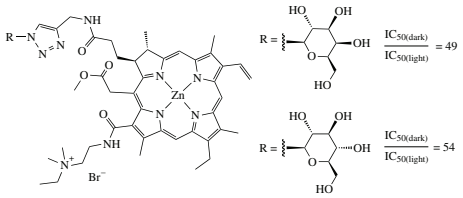
**Natalia S. Kuzmina,[a] Vasilii F. Otvagin,[a] Lubov V. Krylova,[a] Alexander V. Nyuchev,[a] Yuliya V. Romanenko,[b] Oscar I. Koifman,[b] Irina V. Balalaeva[a] and Alexey Yu. Fedorov*[a]**

[a] *N. I. Lobachevsky State University of Nizhny Novgorod, 603950 Nizhny Novgorod, Russian Federation. E-mail: afnn@rambler.ru*

[b] *Research Institute of Macroheterocycles, Ivanovo State University of Chemistry and Technology, 153000 Ivanovo, Russian Federation*

DOI: 10.1016/j.mencom.2020.03.009

**New water soluble conjugates of chlorin $e_6$ derivative–zinc complex with β-D-galactose or β-D-glucose have been synthesized as potential agents for the photodynamic therapy of cancer. They exhibit photoinduced cytotoxicity at low micromolar concentrations with $IC_{50(dark)}/IC_{50(light)}$ ratio of ~50.**




*Keywords*: chlorin $e_6$, glycoconjugate, water soluble, A431 cells, photodynamic therapy.

Photodynamic therapy (PDT) is a promising modality applied to the treatment of cancer and various non-malignant diseases.[1] Anticancer version of PDT is based on utilization of light-activated photosensitizer (PS), which generates reactive oxygen species (ROS), such as singlet oxygen, resulting in a damage to tumor cells.[2]

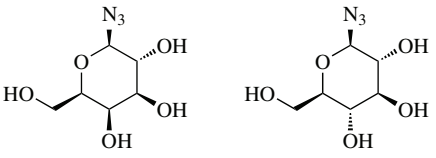
However, up-to-date PSs possess a poor selectivity to tumor tissues, which results in an increased toxicity and a reduced overall therapeutic effect.[3] One common way to tune the selectivity is a creation of conjugated molecules consisting of a photosensitizer and various biological vectors, such as cytostatics,[4–7] monoclonal antibodies,[8] polysaccharides[9,10] and antimicrobial peptides.[11] An alternative approach to provide an edge in the oncoselective delivery consists in the use of nanocarriers like liposomes, micelles or polymers bearing conjugated hybrids of photosensitizers with the oncospecific moieties.[12,13]

Among the hybrid molecules, glycoconjugates are of interest originated from an interaction of carbohydrates with specific proteins, for example galectins, which in turn are known for their expression on tumor cells and demonstrate recognition of sugar moieties.[14,15] Moreover, an accelerated glycolysis inside the cancer cells, known as the Warburg effect, leads to their enhanced sugar uptake.[16] For this reason, glycoconjugated drugs can exhibit cancer-targeted accumulation elevated compared to the unconjugated counterparts.[17,18]

Since naturally occurring chlorin $e_6$ derivatives[19] have low dark cytotoxicity and can efficiently generate singlet oxygen within the phototherapeutic window of 600–700 nm, they stand out as a highly potential platform for creation of the conjugates.[20] However, a chlorin lipophilic core leads to its aggregation in an aqueous medium and, as a consequence, lowered photodynamic activity.[21] Therefore, the conjugation of chlorin $e_6$ with carbohydrate can overcome the poor water solubility and also provide an opportunity to create an amphiphilic molecule, which is an important issue for its biodistribution.[22]

Keeping in mind, that galactose- and glucose-based conjugates are mostly known for improvement of oncoselectivity,[23,24] we have synthesized two new water soluble PDT agents *via* conjugation of zinc-containing chlorin $e_6$ derivative with galactose and glucose as well as investigated their photophysical and biological properties *in vitro*.

Azide-containing saccharides were prepared from commercially available β-D-galactose and β-D-glucose in three steps. The starting monosaccharides were acetylated by $Ac_2O$ in pyridine/DMAP,[25] then azidated using $TMSN_3/SnCl_4$ in $CH_2Cl_2$[26] and finally the acetyl protective groups were cleaved by MeONa in MeOH,[27] affording β-D-galactosyl azide **1a** and β-D-glucosyl azide **1b** in 71 and 78% yields for the three steps, respectively.

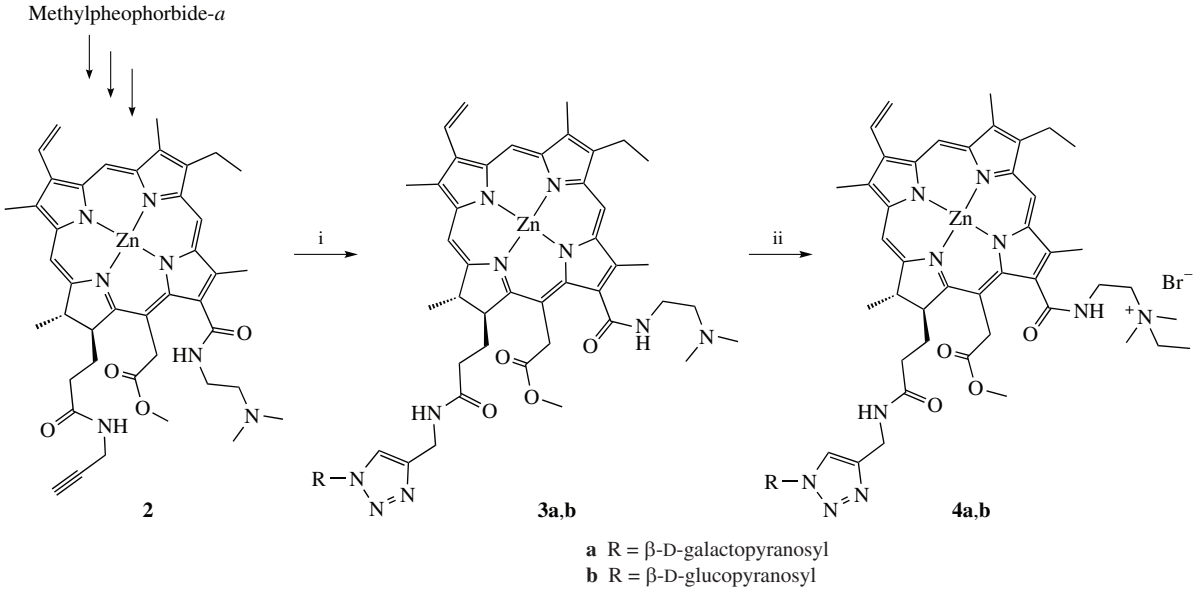


**1a** **1b**

Zinc complexes of chlorins are known for higher quantum yields of singlet oxygen formation compared with their metal-free counterparts.[21] The complex **2** of chlorin $e_6$ derivative with zinc (Scheme 1) was prepared from methylpheophorbide *a* in three steps as described.[5]

Conjugation of glycosyl azides **1a**,**b** with alkyne-containing chlorin **2** *via* copper-mediated 1,3-dipolar cycloaddition[28] using $CuSO_4$/sodium ascorbate catalytic system in the presence of tris(1-benzyltriazol-4-ylmethyl)amine (TBTA) ligand in DMF at 50 °C afforded conjugates **3a** and **3b** in 83 and 69% yields, respectively (see Scheme 1).† To improve the solubility in water,

† *General procedure for the synthesis of compounds* **3a**,**b**. A mixture of $CuSO_4 \cdot 5H_2O$ (0.2 equiv.), sodium ascorbate (0.4 equiv.), TBTA ligand (0.2 equiv.) in water (5 ml) was added dropwise to a solution of glycosyl azide **1a** or **1b** (1.2 equiv.) and chlorin $e_6$ derivative **2** in DMF (5 ml) with stirring. After that the reaction mixture was stirred at 50 °C for 1 h, the solvent was removed *in vacuo* and the product was isolated by column chromatography on silica gel with MeOH–$CHCl_3$ (20 : 80, then 50 : 50).




**Scheme 1** *Reagents and conditions*: i, **1a** or **1b**, TBTA, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, DMF, $H_2O$, 50 °C, 2 h; ii, EtBr, MeOH, $CHCl_3$, room temperature, 48 h.

conjugates **3a**,**b** were converted into the corresponding quaternary ammonium salts by treatment with an excess of ethyl bromide, resulting in compounds **4a**,**b** in quantitative yields.‡ The solubility of conjugates **4a**,**b** was monitored by observation of precipitation from their aqueous solutions for one day. Both products proved to possess solubility of ~$10^{-2}$ mol $dm^{-3}$, which was appropriate for further potential use in the aqueous medium *in vivo*.

Compounds **4a** and **4b** have absorption spectra with intense Soret and Q-bands at ~410 and ~636 nm, respectively (Figure 1). Note that position of their absorption maxima does not depend on the carbohydrate moiety. Both compounds demonstrate fluorescence in water with the maxima in red region at ~644 nm as well as quantum yields of 5.4 and 1.7% relative to Rhodamine 6G for compounds **4a** and **4b**, respectively.

Conjugates **4a**,**b** were incubated with human epidermoid carcinoma A431 cells and demonstrated predominant localization in the intracellular membranes (Figure 2), which can be a result of lipophilic interaction between the chlorin core and the cellular membrane.

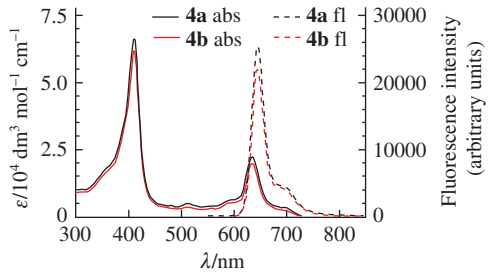



**Figure 1** Absorption and fluorescence spectra ($\lambda_{ex}$ = 410 nm) for 5 μM aqueous solutions of compounds **4a**,**b**.

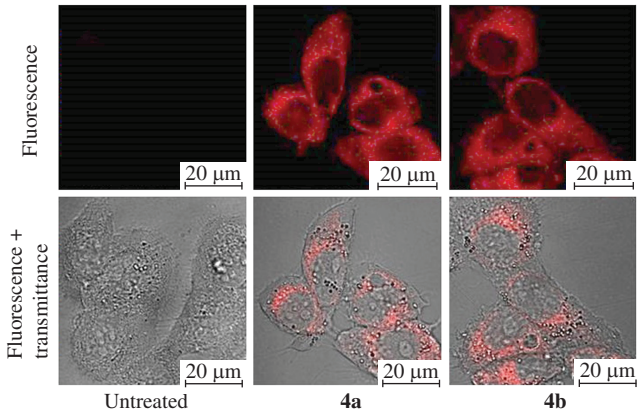



**Figure 2** Confocal images of A431 cells after incubation for 4 h in serum-free growth medium containing compounds **4a**,**b** in 5 μM concentrations. The fluorescence ($\lambda_{ex}$ = 633 nm) was collected in 650–735 nm range.

Photodynamic potency of the synthesized compounds was evaluated using A431 cells by standard MTT assay. Cytotoxicity in the dark along with the cytotoxicity under irradiation with a dose of 20 J $cm^{-2}$ at $\lambda$ = 655–675 nm and power 32 mW $cm^{-2}$ was determined (Table 1). The tested compounds exhibited pronounced dark toxicity at concentrations of 20–25 μM. When the cells were irradiated, photoinduced cytotoxicity was found in low micromolar range with $IC_{50}$ values of 0.47 and 0.41 μM for compounds **4a** and **4b**, respectively. These data demonstrate that the nature of conjugated sugar does not affect *in vitro* activity for the synthesized molecules.

In summary, we have synthesized two water soluble chlorin based conjugates with galactose and glucose. In the *in vitro* studies using A431 cell line the conjugates demonstrated photoinduced cytotoxicity at low micromolar concentrations of ~0.4 μM with $IC_{50(dark)}/IC_{50(light)}$ ratio of ~50 and therefore they could be considered as promising agents for PDT.

**Table 1** $IC_{50}$ values for cytotoxicity of compounds **4a**,**b** to A431 cells.

| Compound | $IC_{50}$/μM | | $IC_{50(dark)}/IC_{50(light)}$ |
|---|---|---|---|
| | Dark | 20 J $cm^{-2}$ | |
| **4a** | 25 | 0.47 | 49 |
| **4b** | 20 | 0.41 | 54 |

*Compound* **3a**. Yield 83%. MS (MALDI), *m*/*z*: 983.6 $[M]^+$ (calc. for $C_{48}H_{59}N_{10}O_9Zn$, *m*/*z*: 983.4).

*Compound* **3b**. Yield 69%. MS (MALDI), *m*/*z*: 983.4 $[M]^+$ (calc. for $C_{48}H_{59}N_{10}O_9Zn$, *m*/*z*: 983.4).

‡ *General procedure for the synthesis of compounds* **4a**,**b**. An excess of ethyl bromide (~300 equiv.) was added to a solution of compound **3a** or **3b** in $CHCl_3$ (2 ml) and MeOH (3 ml). The reaction mixture was stirred at room temperature for 2 days, then the solvent was removed *in vacuo* to give compound **4a** or **4b** in quantitative yield.

For $^1H$ and $^{13}C$ NMR spectra of compounds **3a**,**b** and **4a**,**b**, see Online Supplementary Materials.

This work was supported by the Russian Foundation for Basic Research (project no. 18-33-20041).

*Online Supplementary Materials*

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.03.009.

## References


1 Y.-g. Qiang, X.-p. Zhang, L. Jian and Z. Huang, *Chin. Med. J.*, 2006, **119**, 845.
2 S. B. Brown, E. A. Brown and I. Walker, *Lancet Oncol*., 2004, **5**, 497.
3 Y. Li, J. Wang, X. Zhang, W. Guo, F. Li, M. Yu, X. Kong, W. Wu and Z. Hong, *Org. Biomol. Chem*., 2015, **13**, 7681.
4 L. Liu, R. Wang, C. Wang, J. Wang, L. Chen and J. Cheng, *Biomater. Sci*., 2018, **6**, 997.
5 V. F. Otvagin, A. V. Nyuchev, N. S. Kuzmina, I. D. Grishin, A. E. Gavryushin, Y. V. Romanenko, O. I. Koifman, D. V. Belykh, N. N. Peskova, N. Yu. Shilyagina, I. V. Balalaeva and A. Yu. Fedorov, *Eur. J. Med. Chem.*, 2018, **144**, 740.
6 A. V. Nyuchev, V. F. Otvagin, A. E. Gavryushin, Y. I. Romanenko, O. I. Koifman, D. V. Belykh, H.-G. Schmalz and A. Yu. Fedorov, *Synthesis*, 2015, **47**, 3717.
7 V. F. Otvagin, N. S. Kuzmina, L. V. Krylova, A. B. Volovetsky, A. V. Nyuchev, A. E. Gavryushin, I. N. Meshkov, Y. G. Gorbunova, Y. V. Romanenko, O. I. Koifman, I. V. Balalaeva and A. Yu. Fedorov, *J. Med. Chem.*, 2019, **62**, 11182.
8 M. Mitsunaga, M. Ogawa, N. Kosaka, L. T. Rosenblum, P. L. Choyke and H. Kobayashi, *Nat. Med.*, 2011, **17**, 1685.
9 M. V. Mal'shakova, Y. I. Pylina and D. V. Belykh, *Bioorg. Med. Chem. Lett.*, 2019, **29**, 2064.
10 M. A. Grin, I. S. Lonin, A. I. Makarov, A. A. Lakhina, F. V. Toukach, V. V. Kachala, A. V. Orlova and A. F. Mironov, *Mendeleev Commun.*, 2008, **18**, 135.
11 F. Moret, M. Gobbo and E. Reddi, *Photochem. Photobiol. Sci.*, 2015, **14**, 1238.
12 M. A. Gradova, I. I. Ostashevskaya, O. V. Gradov, A. V. Lobanov, V. S. Lebedeva and A. F. Mironov, *Mendeleev Commun.*, 2018, **28**, 589.
13 A. F. Mironov, K. A. Zhdanova and N. A. Bragina, *Russ. Chem. Rev*., 2018, **87**, 859.
14 S. Singh, A. Aggarwal, N. V. S. D. K. Bhupathiraju, G. Arianna, K. Tiwari and C. M. Drain, *Chem. Rev.*, 2015, **115**, 10261.
15 S. S. Hasan, G. M. Ashraf and N. Banu, *Cancer Lett.*, 2007, **253**, 25.
16 O. Warburg, *Science*, 1956, **123**, 309.
17 M. Wu, H. Li, R. Liu, X. Gao, M. Zhang, P. Liu, Z. Fu, J. Yang, D. Zhang-Negrerie and Q. Gao, *Eur. J. Med. Chem.*, 2016, **110**, 32.
18 R. K. Tekade and X. Sun, *Drug Discovery Today*, 2017, **22**, 1637.
19 H. Tamiaki, S. Takeuchi, S. Tsudzuki, T. Miyatake and R. Tanikaga, *Tetrahedron*, 1998, **54**, 6699.
20 E. Zenkevich, E. Sagun, V. Knyukshto, A. Shulga, A. Mironov, O. Efremova, R. Bonnett, S. P. Songca and M. Kassem, *J. Photochem. Photobiol., B*, 1996, **33**, 171.
21 J. M. Dąbrowski, B. Pucelik, A. Regiel-Futyra, M. Brindell, O. Mazuryk, A. Kyzioł, G. Stochel, W. Macyk and L. G. Arnaut, *Coord. Chem. Rev.*, 2016, **325**, 67.
22 D. V. Titov, M. L. Gening, Yu. E. Tsvetkov and N. E. Nifantiev, *Russ. Chem. Rev.*, 2014, **83**, 523.
23 Y.-S. Lin, R. Tungpradit, S. Sinchaikul, F.-M. An, D.-Z. Liu, S. Phutrakul and S.-T. Chen, *J. Med. Chem.*, 2008, **51**, 7428.
24 H.-F. Liang, C.-T. Chen, S.-C. Chen, A. R. Kulkarni, Y.-L. Chiu, M.-C. Chen and H.-W. Sung, *Biomaterials*, 2006, **27**, 2051.
25 M. A. Flos, M. I. G. Moreno, C. O. Mellet, J. M. G. Fernández, J.-F. Nierengarten and S. P. Vincent, *Chem. Eur. J.*, 2016, **22**, 11450.
26 S. B. Salunke, N. S. Babu and C.-T. Chen, *Chem. Commun.*, 2011, **47**, 10440.
27 M. A. Ameen, S. Karsten, R. Fenger and J. Liebscher, *Tetrahedron Lett.*, 2010, **51**, 4328.
28 J. E. Hein and V. V. Fokin, *Chem. Soc. Rev.*, 2010, **39**, 1302.


*Received: 30th August 2019; Com. 19/6021*