

## Palladium Complexes of Azomethine Derivatives of Porphyrins as Potential Photosensitizers

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*Various isomers of azomethine derivatives of mesoporphyrin IX were first obtained in the form of palladium complexes by the interaction of the so-called "phosphorus complex", produced in situ in the Vilsmeier-Haack reaction of allylamine and methylamine. The compounds were identified and characterized using the methods of NMR 1D and 2D NOESY, and electronic spectroscopy, and mass spectrometry. Photophysical studies of some palladium complexes of meso-(methylimino) and allylimino-derivatives of coproporphyrin I, mesoporphyrin IX and mesochlorin e<sub>6</sub> were carried out. Absorption and photoluminescence (phosphorescence) spectra were studied, and lifetimes of phosphorescence were determined, which were high enough, being several tens of microseconds which corresponds to the previously studied analogous compounds. Peripheral substituents had insignificant effect on the position of the absorption bands and the lifetimes of phosphorescence, therefore, subsequent modification of the azomethine fragment should not significantly change the photophysical properties of the resulting target photosensitizer. The quantum yields of generation of singlet oxygen for the part of the investigated compounds were determined to be quantitative, what implies quantum yield of the triplet excited state of them to be equal to one. The results demonstrate the using prospects of the studied compounds as precursors for subsequent functionalization to obtain potential photosensitizers for photo-oxidation processes with molecular oxygen, in particular, for photodynamic therapy of cancer through the transformation of the azomethine group with the aim of obtaining conjugates of these porphyrins with functional fragments, facilitating efficient transport and selective penetration of photosensitizers in tumor tissue. A preliminary assessment of the photophysical properties of the substances studied allows to position these compounds as precursors for the synthesis of the third generation photosensitizers, which are more suitable for the treatment of superficial cancers due to the shallow penetration of light at the absorption wavelength of these compounds.*

**Keywords:** Photosensitizer, photodynamic therapy, phosphorescence, singlet oxygen, porphyrin, chlorin.

## Палладиевые комплексы азометиновых производных порфиринов как потенциальные фотосенсибилизаторы

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Путем взаимодействия так называемого “фосфорного комплекса”, получаемого *in situ* в реакции Вильсмейера-Хаака с аллиламином и метиламином, впервые синтезированы в виде палладиевых комплексов различные изомеры азометиновых производных мезопорфирина IX. Строение полученных соединений установлено с помощью методов ЯМР спектроскопии 1D и 2D, NOESY, электронной спектроскопии, а также масс-спектрометрии. Проведены фотофизические исследования ряда палладиевых комплексов мезо-(метилимино)- и аллилимино-производных копропорфирина I, мезопорфирина IX и мезохлорина e<sub>6</sub>. Получены спектры поглощения и фотолюминесценции (фосфоресценции), и определены времена жизни фосфоресценции, величины которых достаточно высокие, порядка нескольких десятков микросекунд, что соответствует ранее исследованным аналогичным соединениям. Периферийные заместители слабо влияют как на положение полос поглощения, так и на времена жизни фосфоресценции, следовательно, последующая модификация по азометиновому фрагменту не должна заметно изменить фотофизические свойства получаемых таким образом целевых фотосенсибилизаторов. Определены квантовые выходы генерации синглетного кислорода для части исследованных соединений, которые оказались количественными, что подразумевает квантовый выход триплетного возбужденного состояния для них равным единице. Полученные результаты свидетельствуют о перспективности использования исследуемых соединений в качестве полупродуктов для последующей функционализации с целью получения потенциальных фотосенсибилизаторов для процессов фотоокисления молекулярным кислородом, в частности, для фотодинамической терапии онкологических заболеваний, путем трансформации азометиновой группы для синтеза конъюгатов порфиринов с функциональными фрагментами, способствующими эффективному транспорту и селективному проникновению ФС в опухолевые ткани. Предварительная оценка фотофизических свойств исследованных веществ позволяет позиционировать эти соединения в качестве полупродуктов для получения третьего поколения фотосенсибилизаторов, более подходящих для лечения поверхностного рака вследствие неглубокого проникновения света на длине волны поглощения данных соединений.

**Ключевые слова:** Фотосенсибилизатор, фотодинамическая терапия, фосфоресценция, синглетный кислород, порфирины, хлорины.

## Introduction

Porphyrins and their analogues are widely used in many fields of science and technology, but especially their use is important in medicine. Aromatic electronic system of the tetrapyrrole macrocycle determines their unique electronic and optical properties allowing the use of porphyrins as sensors for diagnostics<sup>[1-2]</sup> and photosensitizers (PS) for the processes of photo-oxidation, in particular, for photodynamic therapy (PDT) of cancer where photosensitized generation of singlet oxygen leads to destruction of tumor tissue during PDT.<sup>[3-10]</sup> It should be noted that the main advantages of PDT are efficiency, ease of operations and absence of severe side effects and diseases compared to standard methods of treatment. However, currently used drugs have certain limitations associated with low rates of accumulation in tumor tissue and prolonged circulation in the human body, which leads to significant skin phototoxicity. Therefore the major problem of PDT is the choice of the optimal structure of the photosensitizer providing maximum treatment efficiency and reduced risk of side effects.<sup>[11]</sup> Publications devoted to the search for new PS appear faster than ever before, and it reflects huge interest of scientists to this field of science. PS based on natural porphyrins and chlorins attract special attention as most efficient drugs for PDT.<sup>[12]</sup> These compounds are similar in structure to those that exist in the human body, therefore, in general principle, they have a relatively low dark toxicity, biodegradable and are easily excreted from the body.<sup>[13]</sup> Asymmetrical structure of chlorin facilitates intensive absorption in red and near IR spectral region, in which the light penetration of tissues is maximal,<sup>[14]</sup> that allows to treat deeply embedded

lesions and pigmented tumors, such as melanoma.<sup>[15]</sup> It is of considerable importance that there is a wide raw material base for these compounds: annual biosynthesis of chlorophyll on our planet is about one billion tons, and the chlorins can be easily extracted from green leaves by simple methods of extraction and precipitation,<sup>[16,17]</sup> and then chemically modified. In this work, Schiff bases were obtained from the corresponding *meso*-formyl derivatives of natural porphyrins and chlorins. Such compounds are interesting because *meso*-imino group can be easily functionalized with the aim to tune and optimize properties of the compound, in particular, to extend conjugation leading to a bathochromic shift in the electronic absorption spectrum, which is important for PDT.

## Experimental

### Materials

Commercial reagents were used without purification, solvents were purified according to standard procedures: CH<sub>2</sub>Cl<sub>2</sub> and 1,2-dichloroethane were distilled over calcium hydride under argon. Palladium complexes of azomethine derivatives of coproporphyrin I **3-6** were prepared according to previously published procedures.<sup>[18,19]</sup> All reactions were performed under protection from direct light under argon and monitored using electronic absorption spectroscopy and by TLC Macherey-Nagel Alugram SIL G/UV254 silica gel 60 UV254. Silica gel 40/60 (Merck) was used for column and flash chromatography. Silica gel 60 (Merck) at 20×20 cm plates with a layer thickness of 1 mm was used for preparative TLC. A mixture of solvents: methylene chloride-methanol was used as an eluent.

## Measurements

$^1\text{H}$  (600 MHz) and  $^{13}\text{C}$  (150 MHz) NMR spectra were recorded on Bruker Avance 600 spectrometer at room temperature and referenced to the residual protons of solvent ( $\text{CDCl}_3$ ;  $\delta_{\text{H}}$  7.28 ppm). MALDI-TOF mass spectra were recorded on an Ultraflex MALDI TOF Bruker Daltonics spectrometer with dithranol matrix. UV-Visible spectra were recorded on a Cary-100 Varian spectrometer. Molecular mechanics MM<sup>+</sup> calculations were performed using *HyperChem* 7.51 program. Electronic spectra were obtained on spectrophotometer HITACHI U-2900. The photoluminescence spectra were recorded on the Fluorolog 3 HORIBA (the exciting light source was xenon lamp with a dual Czerny-Turner monochromator with detection channel Hamamatsu PMT R928).

**Sample preparation.** 5 ml of  $2 \cdot 10^{-6}$  M solution of palladium complex in chloroform were mixed with a solution of 0.5 g of polystyrene (Aldrich) in 10 ml of toluene. The resulting solution was purged with nitrogen to displace dissolved oxygen. The solution was evaporated *in vacuo*, the resulting film of the complex of palladium in polystyrene was dried *in vacuo* to remove traces of solvents.

The phosphorescence in frozen acetone solution at 77 K was measured on a setup with a mechanical phosphoroscope.<sup>[20-22]</sup> Freshly prepared solutions of palladium complexes in acetone were frozen in liquid nitrogen in a special metal frame with a 5 mm thickness. The samples were placed in quartz Dewar vessels filled with liquid nitrogen, secured with special holders inside phosphoroscope. The optical density of solutions of coproporphyrin in 5 mm cuvette was 0.11 at the long-wave absorption maximum (550 nm) before cooling, and that of chlorin solutions was 0.20 at 620 nm. The phosphorescence was excited with focused light of a xenon lamp (1 kW) through a system boundary filters OS-13+ZS-10 (transmittance band 520 nm  $\leq \lambda \leq$  560 nm, measuring the phosphorescence of coproporphyrins) or through a red edge filter KS-11 ( $\lambda = 610$  nm, measuring the phosphorescence of chlorins). Phosphorescence spectra were recorded using a monochromator with a diffraction grating replica. The slit width corresponded to 3.2 nm for coproporphyrins and 10 nm for chlorins.

Quantum yield of singlet oxygen generation was determined by relative method using chemical traps. TPP (*meso*-tetraphenylporphyrin) was used as a standard, for which the quantum yield of singlet oxygen generation was taken equal to  $0.65 \pm 0.05$ .<sup>[23,24]</sup> 1,3-Diphenylisobenzofuran (DPIBF), the interaction of which with singlet oxygen takes place quantitatively with formation of colorless 1,2-dibenzoylbenzene product, was used as a chemical trap.<sup>[25]</sup> For the determination of quantum yields of singlet oxygen generation mixed solutions of the investigated compounds and DPIBF were prepared in a fixed volume of solvent. Measurements were performed in square quartz fluorescence cuvettes with optical path length 1 cm. The solutions were irradiated for a specific time with light passing through the monochromator from a xenon lamp of the fluorimeter Perkin Elmer MPF-44B, light capacity was measured using ThorLabs PM-100D with sensor head S120VC. The wavelength of light, which was used for irradiation of samples, was selected based on the position of the maximum of the red absorption band of the investigated compounds (613 nm). The spectral slit width corresponds to 5 nm. The optical density was determined using a dual beam spectrophotometer Hitachi U-3400. The data were calculated for concentration in the sample corresponding to the optical density equal to 1 in 1 cm cuvette, at the wavelength of DPIBF maximum absorption (412 nm). Calculation of quantum yield by relative method compared the loss of DPIBF optical density and the irradiation time in the experiments performed for the investigated compound and standard compound. Three experiments were performed with each compound.

## Synthesis

**Palladium(II) complex of the diethyl ether of mesoporphyrin IX (1).** 0.113 g (0.18 mmol) of diethyl ester of mesoporphyrin IX were dissolved in 30 ml of acetonitrile, 0.470 g (1.8 mmol) of *trans*-bis(acetonitrile)dichloride palladium(II) were added, and the resulting mixture was refluxed for 3 hours under argon. After the reaction completion the resulting solution was evaporated to dryness in a vacuum rotary evaporator. The obtained residue was dissolved in minimum amount of dry dichloromethane (2–3 ml) and palladium complex of the diethyl ether of mesoporphyrin IX (**1**) has been isolated using column chromatography. Yield: 0.119 g (90 %).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , 25 °C)  $\delta_{\text{H}}$  ppm: 1.14 (6H, t,  $J = 7.7$  Hz,  $\text{CH}_3\text{CH}_2$ ), 1.89 (6H, t,  $J = 7.5$  Hz,  $\text{CH}_3\text{CH}_2\text{O}$ ), 3.31 (4H, q,  $J = 7.3$  Hz,  $\text{CH}_2\text{CH}_2\text{COOEt}$ ), 3.62 (3H, s,  $\beta\text{-CH}_3$ ), 3.63 (3H, s,  $\beta\text{-CH}_3$ ), 3.65 (3H, s,  $\beta\text{-CH}_3$ ), 3.66 (3H, s,  $\beta\text{-CH}_3$ ), 4.06–4.10 (4H, m,  $\text{CH}_2\text{CH}_2\text{COOEt}$ ), 4.16–4.20 (4H, m,  $\text{CH}_2\text{CH}_3$ ), 4.40–4.43 (4H, m,  $\text{CH}_3\text{CH}_2\text{O}$ ), 10.11 (1H, s, CH), 10.12 (1H, s, CH), 10.12 (1H, s, CH), 10.13 (1H, s, CH). UV-Vis ( $\text{CHCl}_3$ )  $\lambda$  nm (lg $\epsilon$ ): 391 (2.54), 511 (1.24), 545 (4.56). Mass spectrum (MALDI TOF)  $m/z$  (%): 727.4 (calcd  $m/z$  for  $[\text{M}+\text{H}]^+$  727.25).

**Palladium(II) complex of the diethyl ether of mesochlorin  $e_6$  (2).** 100 mg (0.16 mmol) of diethyl ether of mesochlorin  $e_6$  was dissolved in 7 ml of benzonitrile, 221 mg (0.47 mmol) of benzonitrile complex of palladium dichloride were added. The reaction mixture was heated under argon atmosphere to 160 °C with constant stirring. 39 mg (0.47 mmol) of sodium acetate were added slowly, in small portions within 5 h. After additional 1 h the reaction mixture was evaporated in vacuum rotary evaporator. Obtained solid residue was purified by thin-layer chromatography on silica gel (eluent: methylene chloride:methanol=25:1). Yield: 112 mg (81 %).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , 25 °C)  $\delta_{\text{H}}$  ppm: 1.69 (3H, t,  $J = 7.8$  Hz,  $8^2\text{-CH}_3$ ), 1.74 (3H, t,  $J = 7.7$  Hz,  $3^2\text{-CH}_3$ ), 1.79 (3H, d,  $J = 7.3$  Hz,  $18\text{-CH}_3$ ), 2.13 (1H, m,  $17^1\text{-CH}_2^a$ ), 2.21 (1H, m,  $17^2\text{-CH}_2^a$ ), 2.25 (1H, m,  $17^1\text{-CH}_2^b$ ), 2.58 (1H, m,  $17^2\text{-CH}_2^b$ ), 3.22 (3H, s,  $7\text{-CH}_3$ ), 3.29 (3H, s,  $2\text{-CH}_3$ ), 3.57 (3H, s,  $12\text{-CH}_3$ ), 3.68 (3H, s,  $17^4\text{-COOCH}_3$ ), 3.75 (2H, q,  $J = 7.8$  Hz,  $8^1\text{-CH}_2$ ), 3.78 (2H, q,  $J = 7.7$  Hz,  $3^1\text{-CH}_2$ ), 3.92 (3H, s,  $15^3\text{-COOCH}_3$ ), 4.28 (3H, s,  $13^2\text{-COOCH}_3$ ), 4.42 (1H, dd,  $J = 10.3$ ,  $J = 2.8$  Hz,  $17\text{-H}$ ), 4.46 (1H, q,  $J = 7.3$  Hz,  $18\text{-H}$ ), 4.98 (1H, d,  $J = 19.7$  Hz,  $15\text{-CH}_2^a$ ), 5.18 (1H, d,  $J = 19.7$  Hz,  $15\text{-CH}_2^b$ ), 8.69 (1H, s,  $20\text{-H}$ ), 9.42 (1H, s,  $5\text{H}$ ), 9.61 (1H, s,  $10\text{-H}$ ). Mass spectrum (MALDI TOF)  $m/z$  (%): 745.2 (calcd  $m/z$  for  $[\text{M}+\text{H}]^+$  745.22). UV-Vis ( $\text{CHCl}_3$ )  $\lambda$  nm (lg $\epsilon$ ): 392 (2.48), 482 (1.16), 565 (1.14), 608 (0.74).

**Synthesis of palladium complexes of meso-methylimino derivatives of mesoporphyrin IX 3a-d under conditions of kinetic control.** Vilsmeier reagent (0.84 g, 3.7 mmol) was added to a solution of 0.05 g (0.069 mmol) of palladium complex of mesoporphyrin IX **1** in 25 ml of dry dichloroethane at 79 °C. The reaction mixture was stirred for 18 hours. After the reaction, the solvent was evaporated *in vacuo*, and 25 ml of distilled water were added to the residue. The organic phase was extracted with dichloromethane and dried over anhydrous sodium sulfate. After filtration and evaporation of the solvent the resulting material was dissolved in dichloromethane (30 ml) and added to the excess of solution of methylamine (28 % solution in water). After completion the reaction mixture was evaporated *in vacuo*, and following isomeric palladium complexes of meso-methylimino derivatives of mesoporphyrin IX were isolated by chromatography: **3a** (0.021 g, 41 %), mixture of isomers **3b+3c** (0.016 g, 32 %), **3d** (0.002 g, 4 %).

**3a:**  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , 25 °C)  $\delta_{\text{H}}$  ppm: 1.20 (6H, t,  $J = 7.9$  Hz,  $\text{CH}_2\text{CH}_3$ ), 1.77 (6H, t,  $J = 7.9$  Hz,  $\text{OCH}_2\text{CH}_3$ ), 3.07 (3H, s,  $\beta\text{-CH}_3$ ), 3.25 (4H, q,  $J = 7.3$  Hz,  $\text{CH}_2$ ), 3.54 (3H, s,  $\beta\text{-CH}_3$ ), 3.51–3.55 (2H, m,  $\text{CH}_2$ ), 3.57 (3H, s,  $\beta\text{-CH}_3$ ), 3.92 (2H, q,  $J = 7.3$  Hz,  $\text{CH}_2$ ), 3.99 (3H, s,  $\text{NCH}_3$ ), 4.19 (4H, m,  $\text{CH}_2$ ), 4.30 (4H, m,  $\text{CH}_2$ ), 9.87 (1H, s, *meso*-H), 9.91 (1H, s, *meso*-H), 9.92 (1H, s, *meso*-H), 10.4 (1H, s,  $\text{CH}=\text{N}$ ). UV-Vis ( $\text{CHCl}_3$ )  $\lambda$  nm (lg $\epsilon$ ): 395 (2.51), 514 (1.14), 548 (0.46). Mass spectrum (MALDI TOF)  $m/z$  (%): 768.4 (calcd  $m/z$  for  $[\text{M}+\text{H}]^+$  768.27).

**3b+3c:**  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , 25 °C)  $\delta_{\text{H}}$  ppm: 1.35–1.38 (18H, m,  $\text{CH}_3$ ), 1.85–1.89 (18H, m,  $\text{CH}_3$ ), 2.86–2.92 (12H, m,  $\text{CH}_2$ ), 3.48 (9H, s,  $\beta$ -Me), 3.52 (9H, s,  $\beta$ -Me), 3.54 (9H, s,  $\beta$ -Me), 3.56 (9H, s,  $\beta$ -Me), 3.82–3.85 (6H, m,  $\text{CH}_2$ ), 2.87–3.90 (6H, m,  $\text{CH}_2$ ), 3.91 (9H, s,  $\text{NCH}_3$ ), 3.99–4.02 (12H, m,  $\text{CH}_2$ ), 4.30–3.34 (12H, m,  $\text{CH}_2$ ), 9.68 (1H, s, *meso*-H), 9.72 (1H, s, *meso*-H), 9.73 (2H, s, *meso*-H), 9.74 (1H, s, *meso*-H), 9.76 (2H, s, *meso*-H), 9.81 (2H, s, *meso*-H), 9.88 (2H, s,  $\text{CH}=\text{N}$ ), 10.03 (1H, s,  $\text{CH}=\text{N}$ ). UV-Vis ( $\text{CHCl}_3$ )  $\lambda$  nm (lg $\epsilon$ ): 396 (2.57), 514 (1.16), 549 (0.47). Mass spectrum (MALDI TOF)  $m/z$  (%): 768.4 (calcd  $m/z$  for  $[\text{M}+\text{H}]^+$  768.27).

**3d:**  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , 25 °C)  $\delta_{\text{H}}$  ppm: 1.35–1.38 (6H, m,  $\text{CH}_3$ ), 1.85–1.89 (6H, m,  $\text{CH}_3$ ), 2.86–2.92 (4H, m,  $\text{CH}_2$ ), 3.48 (3H, s,  $\beta$ - $\text{CH}_3$ ), 3.52 (3H, s,  $\beta$ - $\text{CH}_3$ ), 3.54 (3H, s,  $\beta$ - $\text{CH}_3$ ), 3.56 (3H, s,  $\beta$ - $\text{CH}_3$ ), 3.82–3.85 (2H, m,  $\text{CH}_2$ ), 2.87–3.90 (2H, m,  $\text{CH}_2$ ), 3.91 (3H, s,  $\text{NCH}_3$ ), 3.99–4.02 (4H, m,  $\text{CH}_2$ ), 4.30–3.34 (4H, m,  $\text{CH}_2$ ), 9.91 (1H, s, *meso*-H), 9.95 (2H, s, *meso*-H), 10.52 (1H, s,  $\text{CH}=\text{N}$ ). UV-Vis ( $\text{CHCl}_3$ )  $\lambda$  nm (lg $\epsilon$ ): 395 (2.52), 513–515 (1.15), 549 (0.45). Mass spectrum (MALDI TOF)  $m/z$  (%): 768.4 (calcd  $m/z$  for  $[\text{M}+\text{H}]^+$  768.27).

*Synthesis of palladium complexes of meso-methylimino derivatives of mesoporphyrin IX 3a-d under conditions of kinetic control.* The reaction was carried out similarly to the above procedure for the preparation of palladium complexes of meso-methylimino derivatives of mesoporphyrin IX under conditions of kinetic control, but at temperature 87 °C for 24 hours. The following isomeric meso-methylimino derivatives of mesoporphyrin IX were isolated: **3a** (0.011 g, 21 %), mixture of isomers **3b+3c** (0.022 g, 43 %), **3d** (0.008 g, 15 %).

*Synthesis of palladium complex of meso-methylimino derivative of mesochlorin  $e_6$  (4).* Palladium complex of mesochlorin  $e_6$  **2** (25 mg, 0.047 mmol) was dissolved in 1,2-dichloroethane (20 ml), and the solution was heated to 70 °C. Then Vilsmeier reagent obtained in the reaction of  $\text{POCl}_3$  (0.256 g, 1.68 mmol) and DMF (0.228 g, 1.90 mmol) for 30 minutes at 0 °C was added to the solution. The reaction mixture was heated at 70 °C for 5 hours, then cooled to room temperature and evaporated to dryness *in vacuo*. The obtained oily residue was treated with water and extracted with chloroform (100 ml). The organic layer was evaporated by a half, and methylamine (28 % solution in water, 2 ml) was added and stirred for 5 min. The resulting red solution was evaporated to dryness, and palladium complex of meso-methylimino derivative of mesochlorin  $e_6$  **4** was isolated by column chromatography (silica gel,  $\text{CH}_2\text{Cl}_2:\text{MeOH}=100:1$ ) with 74 % yield.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , 25 °C)  $\delta_{\text{H}}$  ppm: 1.68 (3H, t,  $J=7.8$  Hz,  $8^2\text{-CH}_3$ ), 1.72 (3H, t,  $J=7.7$  Hz,  $3^2\text{-CH}_3$ ), 1.77 (3H, d,  $J=7.3$  Hz,  $18\text{-CH}_3$ ), 2.03 (1H, m,  $17^1\text{-CH}_2^a$ ), 2.17 (1H, m,  $17^2\text{-CH}_2^a$ ), 2.21 (1H, m,  $17^1\text{-CH}_2^b$ ), 2.54 (1H, m,  $17^2\text{-CH}_2^b$ ), 3.18 (3H, c,  $7\text{-CH}_3$ ), 3.25 (3H, c,  $2\text{-CH}_3$ ), 3.51 (3H, c,  $12\text{-CH}_3$ ), 3.64 (3H, c,  $17^4\text{-COOCH}_3$ ), 3.70 (2H, q,  $J=7.8$  Hz,  $8^1\text{-CH}_2$ ), 3.75 (2H, q,  $J=7.7$  Hz,  $3^1\text{-CH}_2$ ), 3.89 (3H, s,  $15^3\text{-COOCH}_3$ ), 4.21 (3H, s,  $13^2\text{-COOCH}_3$ ), 4.38 (1H, dd,  $J=10.3$ ,  $J=2.8$  Hz, 17H), 4.42 (1H, q,  $J=7.3$  Hz, 18H), 4.94 (1H, d,  $J=19.7$  Hz,  $15\text{-CH}_2^a$ ), 5.13 (1H, d,  $J=19.7$  Hz,  $15\text{-CH}_2^b$ ), 8.64 (1H, s, 20-H), 9.38 (1H, s, 5-H), 9.50 (1H, s, 10-H). Mass spectrum (MALDI TOF)  $m/z$  (%): 786.3 (calcd  $m/z$  for  $[\text{M}+\text{H}]^+$  786.25). UV-Vis ( $\text{CHCl}_3$ )  $\lambda$  nm (lg $\epsilon$ ): 403 (2.56), 488 (1.43), 614 (0.84).

*Meso-allylimino derivative of mesochlorin  $e_6$  (5)* was obtained by the same procedure with 72 % yield.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ , 25 °C)  $\delta_{\text{H}}$  ppm: 1.67 (3H, t,  $J=7.9$  Hz,  $8^2\text{-CH}_3$ ), 1.71 (3H, t,  $J=7.7$  Hz,  $3^2\text{-CH}_3$ ), 1.75 (3H, d,  $J=7.2$  Hz,  $18\text{-CH}_3$ ), 2.0 (1H, m,  $17^1\text{-CH}_2^a$ ), 2.22 (1H, m,  $17^2\text{-CH}_2^a$ ), 2.24 (1H, m,  $17^1\text{-CH}_2^b$ ), 2.50 (1H, m,  $17^2\text{-CH}_2^b$ ), 3.15 (3H, s,  $7\text{-CH}_3$ ), 3.23 (3H, s,  $2\text{-CH}_3$ ), 3.49 (3H, s,  $12\text{-CH}_3$ ), 3.61 (3H, s,  $17^4\text{-COOCH}_3$ ), 3.68 (2H, q,  $J=7.8$  Hz,  $8^1\text{-CH}_2$ ), 3.71 (2H, q,  $J=7.7$  Hz,  $3^1\text{-CH}_2$ ), 3.93 (3H, s,  $15^3\text{-COOCH}_3$ ), 4.22 (3H, s,  $13^2\text{-COOCH}_3$ ), 4.35 (1H, dd,  $J=10.1$ ,  $J=2.7$  Hz, 17H), 4.40 (1H, q,  $J=7.2$  Hz, 18H), 4.85 (2H, dd,  $J=6.0$ ,  $J=1.2$  Hz,  $\text{NCH}_2$ ), 4.97 (1H, d,  $J=19.5$  Hz,  $15\text{-CH}_2^a$ ), 5.12 (1H, d,  $J=19.6$  Hz,  $15\text{-CH}_2^b$ ), 5.35 (1H, m,  $J=10.3$ ,  $J=1.8$  Hz,  $\text{NCH}_2\text{CH}=\text{CH}_2$ ), 5.50

(1H, m,  $J=16.2$ ,  $J=1.7$  Hz,  $\text{NCH}_2\text{CH}=\text{CH}_2$ ), 6.42 (1H, dd,  $J=16.5$ ,  $J=10.2$ ,  $J=6.2$  Hz,  $\text{NCH}_2\text{CH}$ ), 8.61 (1H, s, 20-H), 9.35 (1H, s, 5-H), 9.52 (1H, s, 10-H). Mass spectrum (MALDI TOF)  $m/z$  (%): 812.3 (calcd  $m/z$  for  $[\text{M}+\text{H}]^+$  812.26). UV-Vis ( $\text{CHCl}_3$ )  $\lambda$  nm (lg $\epsilon$ ): 401 (2.56), 490 (1.43), 616 (0.84).

## Results and Discussion

For efficient generation of singlet oxygen, PS is to meet the following requirements: high quantum yield and long enough life time of the triplet state. For efficient penetration into tissues irradiating light should be in red or near IR region of the spectrum. Porphyrins can be used as sensitizers in the form of complexes with various metals. The complexation changes the structure of the electronic energy levels of the porphyrin molecule, which allows to tune electronic and optical properties. Heavy metals increase rate of the intersystem crossing  $\text{S1} \Rightarrow \text{T1}$  required for the generation of singlet oxygen. Palladium is especially promising metal center for the porphyrin PS. Palladium is coordination saturated in porphyrin, and therefore there are no complications associated with extracoordination of other molecules to metal center of porphyrin, decreasing the efficiency of singlet oxygen generation. It should be noted that palladium is one of the most accessible and widely used heavy metals in chemistry and industry. Palladium complex of bacteriopheophorbide (WST-09, WST-11) is currently at the last stage of clinical trials as one of the most promising second generation PS Tookad<sup>®</sup>.<sup>[26]</sup> Therefore, we have investigated palladium complexes of meso-imino derivatives of natural porphyrins and chlorins. Imino group is capable of further functionalization, heterocyclization increasing conjugation chain, which can lead to a bathochromic shift of the absorption bands. In this work, the objective was to investigate the photophysical characteristics of palladium complexes of some azomethine derivatives of natural porphyrins, as precursors for creation of the third generation PS.

Mesoporphyrin IX and mesochlorin  $e_6$  were selected as started porphyrins, due to their solubility in aqueous medium enhanced by the presence of carboxyl groups. Azomethine derivatives of palladium complexes of coporphyrin I were obtained as previously reported.<sup>[18,19]</sup> Palladium complexes of azomethine derivatives of mesoporphyrin IX were synthesized for the first time *via* similar procedure. Carboxyl groups were protected by transformation into ethyl esters to avoid complications during functionalization reactions. Then palladium complexes were obtained by reaction with  $\text{Pd}(\text{CH}_3\text{CN})_2\text{Cl}_2$ . Imino derivatives were synthesized by the Vilsmeier-Haack reaction. During formylation of the palladium complex of porphyrins with Vilsmeier reagent, obtained *in situ* by interaction of *N,N*-dimethylformamide and phosphorus oxychloride(V), so called “phosphorus complex” was obtained which was subsequently reacted without isolation with methylamine (Figure 1).<sup>[14]</sup> Four isomeric products were formed in the reaction, the ratio of the isomers has depended on the reaction conditions (Table 1). At lower temperature at conditions close to kinetic control, the reaction mixture was enriched with the product **3a** formed from attack of electrophile at the least sterically hindered position. At higher temperature and longer reaction time the isomers ratio equalize,

indicating the transition to the thermodynamic control of selectivity. For example, sterically hindered isomer **3d** was formed with 12 %, while the yield of the isomer **3a** formed from attack to most accessible position was just 2 % higher. In carrying out the reaction under mild conditions, extremely small amount of **3d** isomer and 40 % yield of the major isomer **3a** were obtained. Chromatographic separation of the reaction mixture has allowed to isolate individual isomers **3a** and **3d**, and a mixture of isomers **3b** and **3c**.

**Table 1.** The ratio of the reaction products of the synthesis of azomethine derivatives of mesoporphyrin IX **3a-d** at various conditions.

	Temperature, °C	Time, h	Yield of reaction products, %		
			<b>3a</b>	<b>3b+3c*</b>	<b>3d</b>
1	87	24	21	43	15
2	79	18	41	32	4

\*Ratio **3b/3c**=1/2

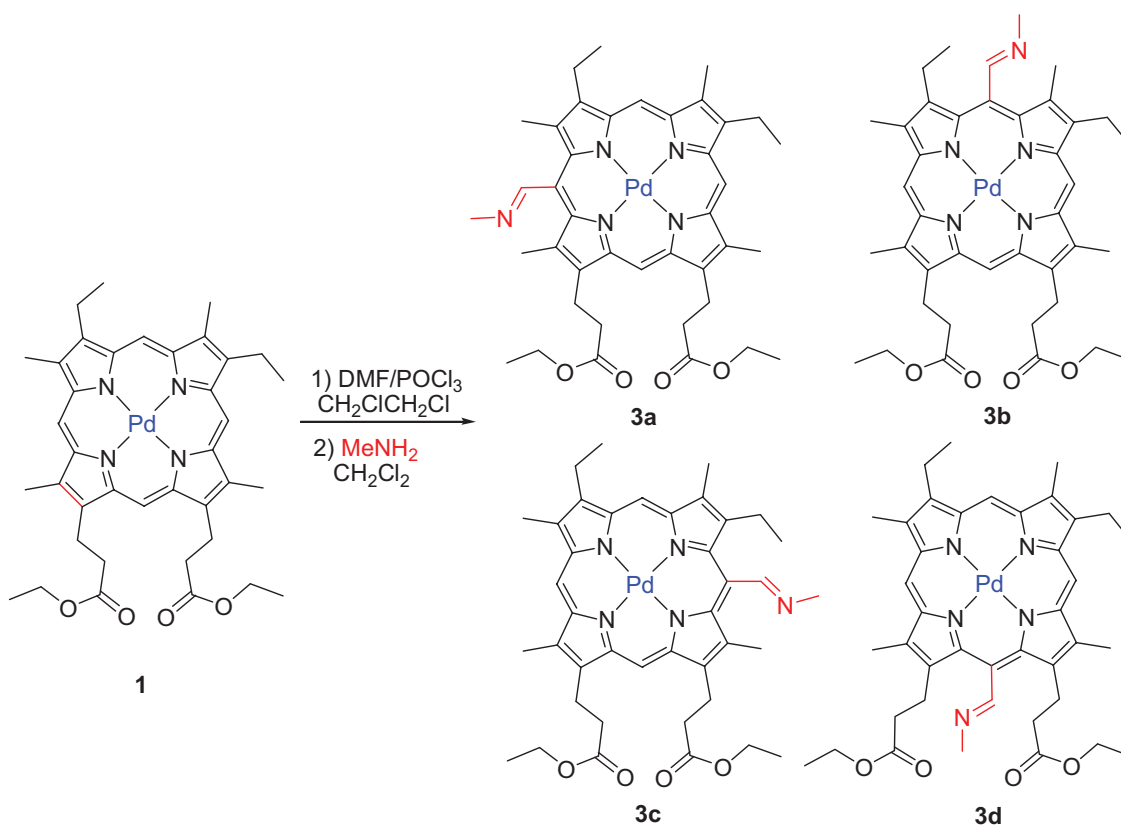
The structure of the reaction products **3a-d** was determined by NMR using NOESY (Supporting information). All azomethine derivatives have *E*-configuration of C=N double bond substituents. There are correlations of C-H proton of the azomethine fragment with  $\beta$ -CH<sub>3</sub> groups in NOESY spectrum confirming the structure of **3a** isomer. NOESY-spectrum of the isomer **3d** contains cross-peaks of the CH=N with the CH<sub>2</sub> protons of the propionic acid fragment.

The earlier developed procedure of the synthesis of Schiff bases of coproporphyrins I and II<sup>[18]</sup> was used to obtain chlorophyll *a* derivative trimethyl ester of mesochlo-

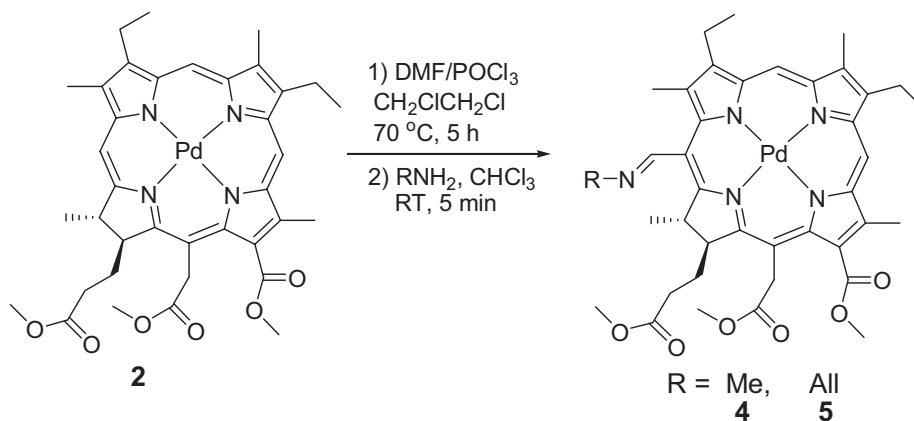
rin *e*<sub>6</sub> (Figure 2). Unlike the porphyrins electrophilic substitution reactions with chlorins were carried out under argon atmosphere because otherwise side reactions were observed, and the yield of the target product did not exceed 20 %.

Electronic absorption spectroscopy has revealed that the modification of *meso*-position with azomethine fragment resulted in a slight bathochromic shift of the absorption bands. Thus, maximum of the Soret band of the palladium complex of the tetraethyl ester methylimino derivative of the coproporphyrin I (**6**) was observed at 392 nm, whereas that of allylimino derivative **7** was at 398 nm, and the positions of the Q-bands have shifted similarly (Table 2). Slightly smaller shifts (3–4 nm) were observed for azomethine derivatives of mesoporphyrin IX **3a-d**. The type of the substituent (methyl, allyl) at imino fragment had little effect on the absorption spectra: the positions of maxima with allyl group **8** and methyl group **9** at azomethine fragment of the derivatives of palladium complex tetraisopropyl ester of coproporphyrin I were almost identical.

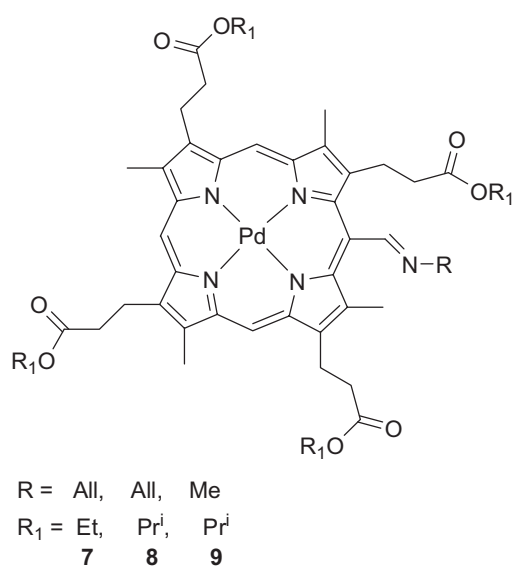
Phosphorescence of the synthesized palladium complexes of azomethine derivatives was investigated in order to assess their potential photosensitizing ability. Excitation of compounds **3a-d** and **7** was carried out at the wavelength of the Soret band 397 nm. The time dependence of the phosphorescence was recorded at maximum of phosphorescence at 670 nm. Figure 4 shows the photoluminescence spectrum of **3d**. Along with the main maximum of the phosphorescence a satellite maximum at 728 nm with intensity ~10 % of the main was observed. Similar minor peaks or shoulders were observed at the spectra of the other compounds (Figure 5). The energy of the triplet states of the studied compounds was significantly higher than singlet <sup>1</sup>Δ<sub>g</sub> state of oxygen, which



**Figure 1.** Scheme of synthesis of azomethine derivatives of mesoporphyrin IX **3a-d**.



**Figure 2.** Scheme of synthesis of azomethine derivatives of mesochlorin *e*<sub>6</sub> 4, 5.

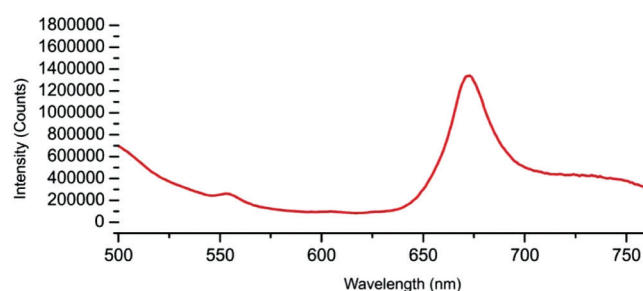


**Figure 3.** Azomethine derivatives of coproporphyrin I 7-9.

facilitates energy transfer from the excited PS molecule to molecule O<sub>2</sub>. Palladium complexes of azomethine derivatives of mesochlorin *e*<sub>6</sub> 4, 5 possess the longest absorption wavelength of 616 nm at the maximum of Q<sub>y</sub> band of the studied compounds. Accordingly, the longest wavelength maximum of the phosphorescence at 822 nm was observed for compound 5, and the spectrum of compound 4 had a close maximum at 810 nm (Table 3). The lifetimes of the phosphorescence of the investigated compounds were of the same order, 70 μs on average, except for mesochlorin 4, which was two times less than the average. It can be seen from the data in Table 3 that the peripheral substituents have little effect on the position of the absorption bands and the lifetimes of phosphorescence, therefore, subsequent modification at the azomethine fragment should not significantly change the photophysical properties of the resulting target PS. A preliminary assessment allows to position the investigated compounds as precursors for PS, which are more suitable for the treatment of superficial cancers due to the shallow penetration of light at the absorption wavelength of these compounds.

**Table 2.** Maxima of the absorption bands in porphyrin electronic spectra (in CHCl<sub>3</sub>).

Compound	Absorption bands, nm		
	Soret	Q <sub>x</sub>	Q <sub>y</sub>
1	391	511	545
2	391	482	608
3a	395	514	548
3d	396	514	549
3b,c	395	514	549
4	403	488	614
5	401	490	616
6	392	511	545
7	398	516	549
8	398	516	549
9	397	516	549



**Figure 4.** The photoluminescence spectrum of 3d in polystyrene matrix.

Quantum yield of generation of singlet oxygen was determined for compounds 4, 5, 8, 9. For this purpose we have applied a relative method using chemical trap 1,3-diphenylisobenzofuran (DPIBF). The standard compound was TPP (*meso*-tetraphenylporphin), for which the quantum yield of singlet oxygen was assumed to be 0.65 ± 0.05.<sup>[23,24]</sup> Porphyrins were irradiated with light with a wavelength corresponding to the Q<sub>y</sub> absorption band. Change of optical density was

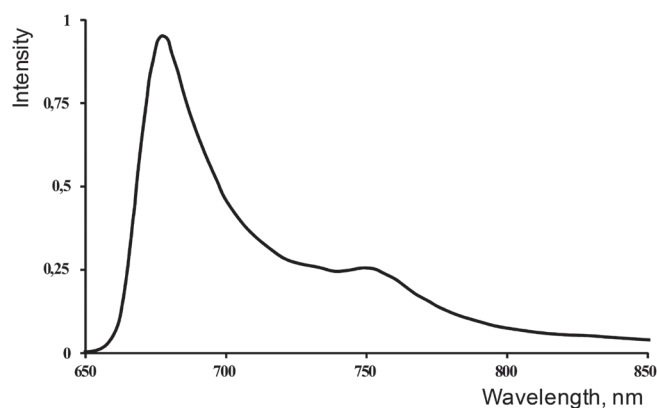


Figure 5. The phosphorescence spectrum of **9** in acetone at 77 K.

controlled at irradiation not only at the absorption maximum of DPIBF, but also at the wavelength of the exciting light. During the irradiation the absorption band of the sensitizer did not fade, *i.e.* the optical density of the sensitizer at the wavelength of the exciting light did not change. The details of the calculation are given in Supporting information. Quantum yield of singlet oxygen generation  $\phi$  was 100 % for all four investigated porphyrins (Table 3). This result was quite expected, given an enough large life time of the phosphorescence, for which the oxygen had time to diffuse to the excited PS molecule, and a sufficiently large difference in the energies of the excited triplet state of the PS and singlet oxygen. Quantitative yield of generation of singlet oxygen assumes quantum yield of the triplet state of palladium complexes of porphyrins equal to one. Thus, the investigated azomethine derivatives of porphyrins **3–5**, **7–9** can be promising precursors for development the third generation PS for PDT on their basis. Further modification of these substances can be performed by transformation of the azomethine group with the aim of obtaining conjugates of these porphyrins with functional fragments, facilitating efficient transport and selective penetration of PS in tumor tissue.

## Conclusions

The results of the study of palladium complexes of porphyrins azomethine derivatives have revealed that *meso*-

methylimino and allylimino derivatives of mesochlorin **6**, **4**, **5** and coproporphyrin I **8**, **9** possess the photophysical characteristics required for their successful use as PS in PDT, namely, long life time of phosphorescence at the order of several tens of microseconds, and quantitative quantum yield of the triplet state of metal porphyrins, and singlet oxygen generation. Thus, these compounds can be promising basis for development of efficient PS by means of further functionalization of azomethine fragment for the purpose of tuning their properties as drug substance such as tissue distribution, metabolism, and dark toxicity. Relevant studies are in progress and will be published later.

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Table 3. Data of photophysical studies of porphyrins.

Compound	Maxima of bands, nm		Phosphorescence life time, $\mu$ s	Quantum yield of singlet oxygene $\phi$
	Fluorescence	Phosphorescence		
<b>3a</b>	552	671	84	
<b>3d</b>	557	672	71	
<b>3bc</b>	555	671	72	
<b>7</b>	554	671	84	
<b>4</b>		810	35	1.00 $\pm$ 0.07
<b>5</b>		822	80	1.00 $\pm$ 0.07
<b>8</b>		684	81	1.00 $\pm$ 0.05
<b>9</b>		678	65	1.00 $\pm$ 0.05

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