Палладиевые комплексы азометиновых производных порфиринов как потенциальные фотосенсибилизаторы

В. С. Тюрин[a], Д. Р. Эрзина[a], И. А. Замилацков[a@], А. Ю. Чернядьев[a], Г. В. Пономарев[b], Д. В. Яшунский[b], А. В. Максимова[a], К. В. Неверов[c], А. С. Козлов[c], А. А. Красновский[c], А. Ю. Цивадзе[a]

[a]*Институт физической химии и электрохимии им. А.Н. Фрумкина РАН, 119071, Москва, Россия*
[b]*Научно-исследовательский Институт биомедицинской химии им. В.Н. Ореховича РАМН, 119121, Москва, Россия*
[c]*Федеральный Исследовательский Центр «Фундаментальные основы биотехнологии» РАН, Институт биохимии им. А.Н. Баха, 119071, Москва, Россия*
[@]*E-mail: joz@mail.ru*

Путем взаимодействия так называемого “фосфорного комплекса”, получаемого *in situ* в реакции Вильсмейера-Хаака с аллиламином и метиламином, впервые получены в виде палладиевых комплексов различные изомеры азометиновых производных мезопорфирина IX. Строение полученных соединений установлено с помощью электронных спектров поглощения и методов ЯМР спектроскопии 1D и 2D, NOESY, а также масс-спектрометрии. Исследованы фотофизические параметры (спектры поглощения, флуоресценции и фосфоресценции, время жизни фосфоресценции и квантовый выход генерации синглетного кислорода) для ряда палладиевых комплексов *мезо*-(метилимино)- и аллилимино-производных копропорфирина I, мезопорфирина IX и мезохлорина e_6. Показано, что время жизни фосфоресценции порфиринов в пленке полистирола составляет 71-84 мкс при комнатной температуре и 0,35-2 мс при 77 К в ацетоне. Энергия триплетного состояния соответствует энергии фотонов с длиной волны 670-820 нм. Квантовый выход генерации синглетного кислорода в аэробных растворах порфиринов при комнатной температуре близок к единице. Полученные результаты свидетельствуют о перспективности использования исследуемых соединений в качестве полупродуктов для получения фотодинамических сенсибилизаторов (фотосенсибилизаторов, ФС) третьего поколения, пригодных для лечения поверхностных раковых опухолей. В частности, трансформация азометиновой группы может использоваться для синтеза конъюгатов порфиринов с функциональными фрагментами, способствующими эффективному транспорту и селективному проникновению ФС в опухолевые клетки.

Ключевые слова: фотосенсибилизатор, фотодинамическая терапия, фосфоресценция, синглетный кислород, порфирин, хлорин.

Palladium Complexes of Azomethine Derivatives of Porphyrins as Potential Photosensitizers

V. S. Tyurin[a], D. R. Erzina[a], I. A. Zamilatskov[a@], A.Yu. Chernyadiev[a], G. V. Ponomarev[b], D. V. Yashunskiy[b], A. V. Maksimova[a], K. V. Neverov[c], A. S. Kozlov[c], A. A. Krasnovsky[c], A. Yu. Tsivadze[a]

[a]*Frumkin Institute of Physical Chemistry and Electrochemistry, Russian Academy of Sciences, 119071, Moscow, Russian Federation*
[b]*Research Institute of Biomedical Chemistry, Russian Academy of Medical Sciences, 119121, Moscow, Russian Federation*
[c]*Federal Research Centre "Fundamentals of Biotechnology" of the Russian Academy of Sciences, Bach Institute of Biochemistry, 119071, Moscow, Russian Federation*
[@]*E-mail: joz@mail.ru*

Various isomers of azomethine derivatives of mesoporphyrin IX were obtained for the first time 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 electronic absorption spectroscopy, NMR 1D and 2D, NOESY and mass spectrometry. Photophysical parameters (absorption, fluorescence and phosphorescence spectra, phosphorescence lifetimes and the quantum yields of singlet oxygen generation) were studied for palladium complexes of *meso*-(methylimino) and allylimino-derivatives of coproporphyrin I, mesoporphyrin IX and mesochlorin e_6. It was shown that the phosphorescence lifetime of all compounds was 71-84 μs at room temperature in solid films of polystyrene and within 0.35-2 ms in frozen solutions in acetone at 77 K. The energy of triplet states corresponded to the energy of photons with the wavelengths 670-820 nm. The quantum yields of singlet oxygen generation by all porphyrins were shown to be close to unity. The results indicate that the investigated compounds can be used as precursors for synthesis of the photodynamic sensitizers (photosensitizers, PS) of the third generation suitable for the treatment of superficial cancer tumors. Transformation of their azomethine groups might be applied for synthesis of new conjugates with functional fragments facilitating efficient transport and selective penetration of PS in tumor cells.

Keywords: photosensitizer, photodynamic therapy, phosphorescence, singlet oxygen, porphyrin, chlorin.

Introduction

Porphyrins and their analogues are widely used in many fields of science and technology, but especially important is their use 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[1-2] 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.[3-10] 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.[11] 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.[12] These compounds are similar in structure to those that exist in the human body, therefore, in general principle, have a relatively low dark toxicity, biodegradable and are easily excreted from the body.[13] Asymmetrical structure of chlorin facilitates intensive absorption in red and near IR spectral region in which the light penetration of tissues is maximal,[14] that allows to treat deeply embedded lesions and pigmented tumors, such as melanoma.[15] 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 easily be extracted from green leaves by simple methods of extraction and precipitation[16,17] and then chemically modified. In this work, Schiff bases were obtained from the corresponding of *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_2Cl_2 and 1,2-dichloroethane were distilled over calcium hydride under argon. Palladium complexes of azomethine derivatives of coproporphyrin I **7**-**9** were prepared according to previously published procedures.[18,19] 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/UV_{254} silica gel 60 UV_{254}. Silica gel 40/60 (Merck) was used for column and flash chromatography. Silica gel 60 (Merck) at 20x20 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

^1H (600 MHz) and ^{13}C (150 MHz) NMR spectra were recorded on Bruker Avance 600 spectrometer at room temperature and referenced to the residual protons of solvent ($CDCl_3$ – δ 7.28 ppm). MALDI-TOF mass spectra were recorded on an Ultraflex MALDI TOF Bruker Daltonics spectrometer with dithranol matrix. Molecular mechanics MM^+ calculations were performed by using HyperChem v. 7.51 software.[27] Electronic absorption spectra were obtained on spectrophotometers Hitachi U-2900, Varian Cary-100 and SF-56. The room temperature fluorescence and phosphorescence spectra were recorded on the Horiba Fluorolog 3 fluorimeter (the exciting light source was xenon lamp with a dual Czerny-Turner monochromator with detection channel Hamamatsu PMT R928). For room temperature phosphorescence measurements porphyrins were incorporated into polystyrene films using the following procedure: 5 ml of 2 μM solution of palladium complex in chloroform was 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 and then evaporated in vacuo. The resulting film was dried in vacuo to remove traces of solvents. Phosphorescence decays were detected upon excitation by flash xenon lamp using the technique of delays changing after flashes, and analyzed by FluorEssenceTM software for the lifetime calculation.

The phosphorescence in frozen acetone solution at 77 K was measured on a setup with a mechanical phosphoroscope.[20-22] Freshly prepared solutions of palladium complexes in acetone were frozen in liquid nitrogen in a special metal frame with thickness of 5 mm. 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 solutions of chlorin was 0.20 at 620 nm. The phosphorescence was excited with focused light of a xenon

lamp (1 kW) through a system of boundary filters OS-13 + ZS-10 (transmittance band 520 nm ≤ λ ≤ 560 nm, measuring the phosphorescence of coproporphyrins) or through a red edge filter KS-11 (λ = 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*-tetraphenylporphin) was used as a standard, for which the quantum yield of singlet oxygen generation was taken equal to 0.65 ± 0.05.[23,24] 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.[25] 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 of 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 for 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 was dissolved in 30 ml of acetonitrile, 0.470 g (1.8 mmol) of trans-bis(acetonitrile)dichloride palladium (II) was 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 in 0.119 g (90%) yield. δ_H ($CDCl_3$, 298 K) 1.14 (6H, t, *J* = 7.7, $\underline{CH_3}CH_2$), 1.89 (6H, t, *J* = 7.5, $\underline{CH_3}CH_2O$), 3.31 (4H, q, *J* = 7.3, $CH_2\underline{CH_2}COOEt$), 3.62 (3H, s, β-$CH_3$), 3.63 (3H, s, β-$CH_3$), 3.65 (3H, s, β-$CH_3$), 3.66 (3H, s, β-$CH_3$), 4.06-4.10 (4H, m, $\underline{CH_2}CH_2COOEt$), 4.16-4.20 (4H, m, $\underline{CH_2}CH_3$), 4.40-4.43 (4H, m, $CH_3\underline{CH_2}O$), 10.11 (1H, s, CH), 10.12 (1H, s, CH), 10.12 (1H, s, CH), 10.13 (1H, s, CH). λ_{max} ($CHCl_3$) (logε)/nm 391 (2.54), 511 (1.24), 545 (4.56). MALDI ToF MS: m/z 727.4, 725.4, 726.4, 728.4, 729.4, 730.4, 731.4 (calcd m/z for $[M+H]^+$ 727.25).

*Palladium (II) complex of the diethyl ether of mesochlorin e6 **2***. 100 mg (0.16 mmol) of diethyl ether of mesochlorin *e6* was dissolved in 7 ml of benzonitrile. 221 mg (0.47 mmol) of benzonitrile complex of palladium dichloride was added. The reaction mixture was heated under argon atmosphere to 160°C with constant stirring. 39 mg (0.47 mmol) of sodium acetate was added slowly, in small portions within 5 h. After additional 1 h the reaction mixture was evaporated on vacuum rotary evaporator. Obtained solid residue was purified by thin-layer chromatography on silica gel with eluent methylene chloride/methanol 25:1 yielding 112 mg (81%) of **2**. δ_H ($CDCl_3$, 298 K) 1.69 (3H, t, *J* = 7.8, 8^2-CH_3), 1.74 (3H, т, *J* = 7.7, 3^2- CH_3), 1.79 (3H, д, *J* = 7.3, 18-CH_3), 2.13 (1H, м, 17^1- CH_2^a), 2.21(1H, м, 17^2- CH_2^a), 2.25 (1H, m, 17^1-CH_2^b), 2.58 (1H, m, 17^2-CH_2^b), 3.22 (3H, s, 7-CH_3), 3.29 (3H, s, 2-CH_3), 3.57 (3H, s, 12-CH_3),

3.68 (3H, s, 17^4-$COOCH_3$), 3.75 (2H, q, *J* = 7.8, 8^1-CH_2), 3.78 (2H, q, *J* = 7.7, 3^1-CH_2), 3.92 (3H, s, 15^3-$COOCH_3$), 4.28 (3H, s, 13^2-$COOCH_3$), 4.42(1H, dd, *J* = 10.3, *J* = 2.8, 17-H), 4.46 (1H, q, *J* = 7.3, 18-H), 4.98 (1H, d, *J* = 19.7, 15-CH_2^a), 5.18 (1H, d, *J* = 19.7, 15-CH_2^b), 8.69 (1H, s, 20-H), 9.42 (1H, s, 5H), 9.61 (1H, s, 10-H). MALDI ToF MS: m/z 745.2, 743.2, 744.2, 745.2, 746.2, 747.2, 748.2, 749.2 (calcd m/z for $[M+H]^+$ 745.22). λ_{max} ($CHCl_3$) (logε)/nm 392 (2.48), 482 (1.16), 565 (1.14), 608 (0.74).

*Synthesis of palladium (II) 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 dry dichloroethane at 79 ºC. The reaction mixture was stirred for 18 hours. After the reaction, the solvent was evaporated in a vacuum and 25 ml of distilled water was 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 reaction 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%).

3*a*: δ_H ($CDCl_3$, 298 K) 1.20 (6H, t, *J* = 7.9, $CH_2\underline{CH_3}$), 1.77 (6H, t, *J* = 7.9, $OCH_2\underline{CH_3}$), 3.07 (3H, s, β-CH_3), 3.25 (4H, q, *J* = 7.3, CH_2), 3.54 (3H, s, β-CH_3), 3.51-3.55 (2H, m, CH_2), 3,57 (3H, s, β-CH_3), 3.92 (2H, q, *J* = 7.3, CH_2), 3.99 (3H, s, NCH_3), 4.19 (4H, m, CH_2), 4.30 (4H, m, CH_2), 9.87 (1H, s, *meso*-H), 9.91 (1H, s, *meso*-H), 9.92 (1H, s, *meso*-H), 10.4 (1H, s, CH=N). λ_{max} ($CHCl_3$) (logε)/nm 395 (2.51), 514 (1.14), 548 (0.46). MALDI ToF MS: m/z 768.4, 766.4, 767.4, 768.4, 769.4, 770.4, 771.4, 772.4 (calcd m/z for $[M+H]^+$ 768.27).

3*b*+*3c*: δ_H ($CDCl_3$, 298 K) 1.35-1.38 (18H, m, CH_3), 1.85-1.89 (18H, m, CH_3), 2.86-2.92 (12H, m, CH_3), 3.48 (9H, s, β-Me), 3.52 (9H, s, β-Me), 3.54 (9H, s, β-Me), 3.56 (9H, s, β-Me), 3.82-3.85 (6H, m, CH_2), 2.87-3.90 (6H, m, CH_2), 3.91 (9H, s, NCH_3), 3.99-4.02 (12H, m, CH_2), 4.30-3.34 (12H, m, 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, CH=N), 10.03 (1H, s, CH=N). λ_{max} ($CHCl_3$) (logε)/nm 396 (2.57), 514 (1.16), 549 (0.47). MALDI ToF MS: m/z 768.4, 766.4, 767.4, 768.4, 769.4, 770.4, 771.4, 772.4 (calcd m/z for $[M+H]^+$ 768.27).

3*d*: δ_H ($CDCl_3$, 298 K) 1.35-1.38 (6H, m, CH_3), 1.85-1.89 (6H, m, CH_3), 2.86-2.92 (4H, m, CH_2), 3.48 (3H, s, β-CH_3), 3.52 (3H, s, β-CH_3), 3.54 (3H, s, β-CH_3), 3.56 (3H, s, β-CH_3), 3.82-3.85 (2H, m, CH_2), 2.87-3.90 (2H, m, CH_2), 3.91 (3H, s, NCH_3), 3.99-4.02 (4H, m, CH_2), 4.30-3.34 (4H, m, CH_2), 9.91(1H, s, *meso*-H), 9.95 (2H, s, *meso*-H), 10.52 (1H, s, CH=N). λ_{max} ($CHCl_3$) (logε)/nm 395 (2.52), 513-515 (1.15), 549 (0.45). MALDI ToF MS: m/z 768.4, 766.4, 767.4, 768.4, 769.4, 770.4, 771.4, 772.4 (calcd m/z for $[M+H]^+$ 768.27).

*Synthesis of palladium (II) 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 ^{0}C for 24 hours. Following isomeric *meso*-methylimino derivatives of mesoporphyrin IX were isolated: **3*a*** (0.011 g, 21%)**,** mixture of isomers **3b**+**3c** (0.022 g, 43%), **3*d*** (0.008 g, 15%).

*Synthesis of palladium (II) 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 ºC. Then Vilsmeier reagent obtained in

the reaction of $POCl_3$ (0.256 g, 1.68 mmol) and DMF (0.228 g, 1.90 mmol) for 30 minutes at 0 ºC was added to the solution. The reaction mixture was heated at 70 ºC for 5 hours, then cooled to room temperature and evaporated to dryness at vacuum. The obtained oily residue was treated with water and extracted with chloroform (100 ml). The organic layer was evaporated by 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, CH_2Cl_2/MeOH 100:1) with 74% yield. δ_H ($CDCl_3$, 298 K) 1.68 (3H, t, J = 7.8, 8^2-CH_3), 1.72 (3H, t, J = 7.7, 3^2- CH_3), 1.77 (3H, d, J = 7.3, 18-CH_3), 2.03 (1H, m, 17^1- CH_2^a), 2.17 (1H, m, 17^2- CH_2^a), 2.21 (1H, m, 17^1- CH_2^b), 2.54 (1H, m, 17^2-CH_2^b), 3.18 (3H, c, 7-CH_3), 3.25 (3H, c, 2-CH_3), 3.51 (3H, c, 12-CH_3), 3.64 (3H, c, 17^4-$COOCH_3$), 3.70 (2H, q, J = 7.8, 8^1-CH_2), 3.75 (2H, q, J = 7.7, 3^1-CH_2), 3.89 (3H, s, 15^3-$COOCH_3$), 4.21 (3H, s, 13^2-$COOCH_3$), 4.38 (1H, dd, J = 10.3, J = 2.8, 17H), 4.42 (1H, q, J = 7.3, 18H), 4.94 (1H, d, J = 19.7, 15-CH_2^a), 5.13 (1H, d, J = 19.7, 15-CH_2^b), 8.64 (1H, s, 20-H), 9.38 (1H, s, 5-H), 9.50 (1H, s, 10-H). MALDI ToF MS: m/z 786.3, 784.3, 785.3, 787.3, 788.3, 789.3, 790.3 (calcd m/z for $[M+H]^+$ 786.25). λ_{max} ($CHCl_3$) (logε)/nm 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. δ_H ($CDCl_3$, 298 K) 1.67 (3H, t, J =7.9, 8^2-CH_3), 1.71 (3H, t, J = 7.7, 3^2- CH_3), 1.75 (3H, d, J = 7.2, 18-CH_3), 2.0 (1H, m, 17^1- CH_2^a), 2.22 (1H, m, 17^2- CH_2^a), 2.24 (1H, m, 17^1- CH_2^b), 2.50 (1H, m, 17^2-CH_2^b), 3.15 (3H, s, 7-CH_3), 3.23 (3H, s, 2-CH_3), 3.49 (3H, s, 12-CH_3), 3.61 (3H, s, 17^4-$COOCH_3$), 3.68 (2H, q, J = 7.8, 8^1-CH_2), 3.71 (2H, к, J = 7.7, 3^1-CH_2), 3.93 (3H, c, 15^3-$COOCH_3$), 4.22 (3H, c, 13^2-$COOCH_3$), 4.35 (1H, dd, J = 10.1, J = 2.7, 17H), 4.40 (1H, q, J = 7.2, 18H), 4.85 (2H, dd, J = 6.0, J = 1.2, NCH_2), 4.97 (1H, d, J = 19.5, 15-CH_2^a), 5.12 (1H, d, J = 19.6, 15-CH_2^b), 5.35 (1H, m, J = 10.3, J = 1.8, $NCH_2CH=\underline{CH_2}$), 5.50 (1H, м, J = 16.2, J = 1.7, $NCH_2CH=\underline{CH_2}$), 6.42 (1H, ddt, J = 16.5, J = 10.2, J = 6.2, $NCH_2\underline{CH}$), 8.61 (1H, s, 20-H), 9.35 (1H, s, 5-H), 9.52 (1H, s, 10-H). MALDI ToF MS: m/z 812.3, 810.3, 812.3, 811.3, 813.3, 814.3, 815.3, 816.3 (calcd m/z for $[M+H]^+$ 812.26). λ_{max} ($CHCl_3$) (logε)/nm 401 (2.56), 490 (1.43), 616 (0.84).

Results and discussions

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 S_1=>T_1 required for the generation of singlet oxygen. Palladium is especially promising metal center for the porphyrin PS. Palladium is coordinatively 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 available 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®.[26] Therefore, we have investigated palladium complexes of *meso*-iminoderivatives 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 initial porphyrins, due to their solubility in aqueous medium enhanced by the presence of carboxyl groups. Azomethine derivatives of palladium complexes of coproporphyrin I were obtained as previously reported.[18,19] 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 $Pd(CH_3CN)_2Cl_2$. 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 (Fig. 1).[14] Four isomeric products were formed in the reaction, the ratio of the isomers 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 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, %		
			3a	**3b+3c***	**3d**
1	87	24	21	43	15
2	79	18	41	32	4

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

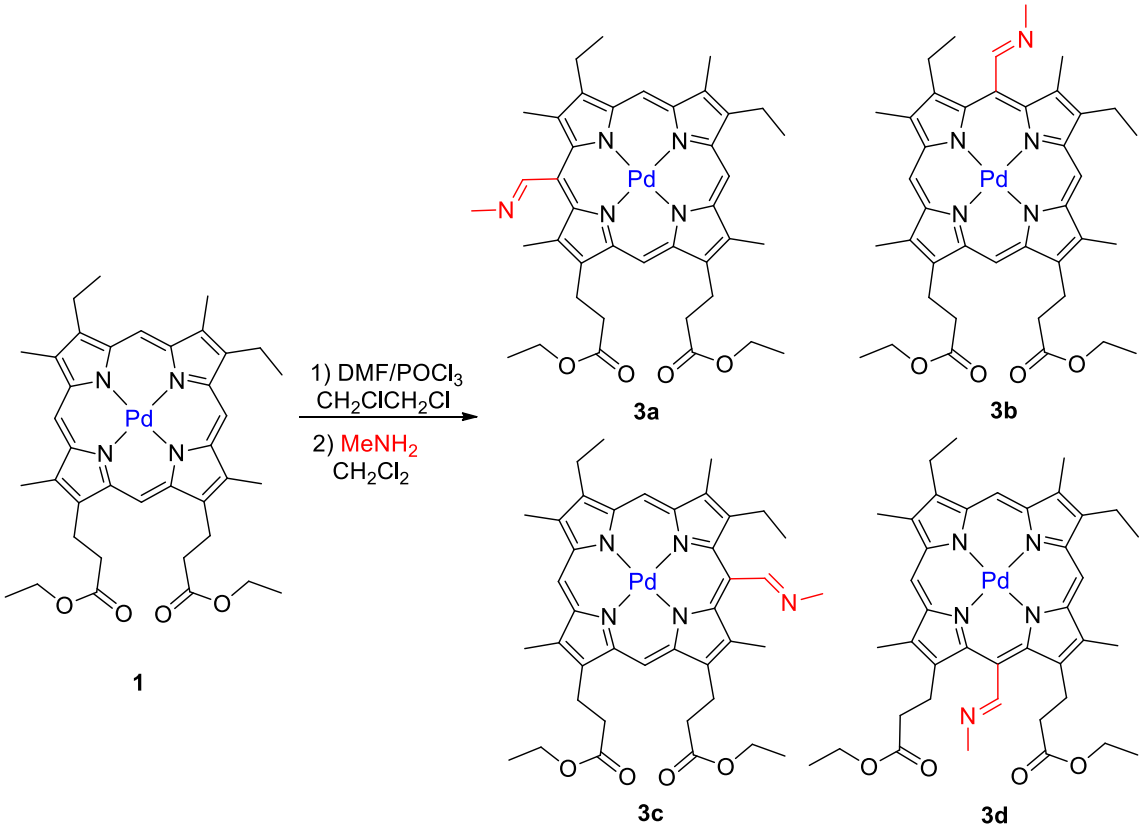

Figure 1. Scheme of synthesis of azomethine derivatives of mesoporphyrin IX **3a-d**.

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 β-CH_3 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_2 protons of the propionic acid fragment.

The earlier developed procedure of the synthesis of Schiff bases of coproporphyrins I and II[18] was used to obtain chlorophyll a derivative trimethyl ester of mesochlorin e_6 (Fig. 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%.

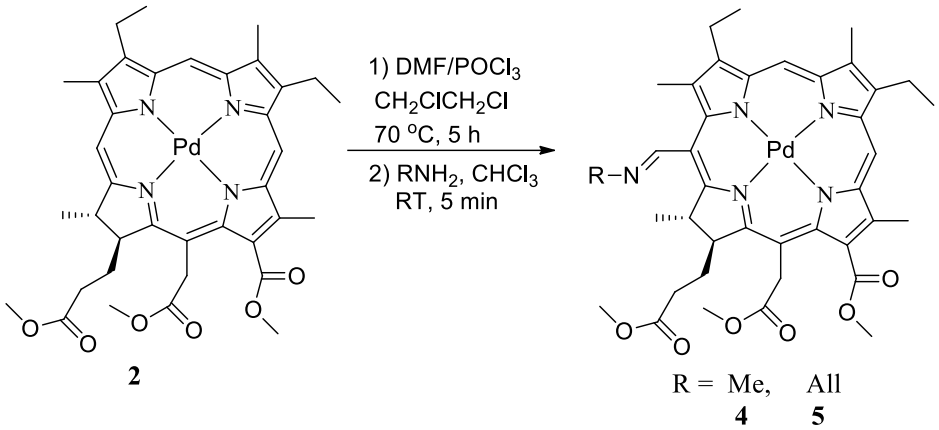

Figure 2. Scheme of synthesis of azomethine derivatives of mesochlorin e_6 **4**, **5**.

Electronic absorption spectroscopy 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 methylimino derivative of the tetraethyl ester 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 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.

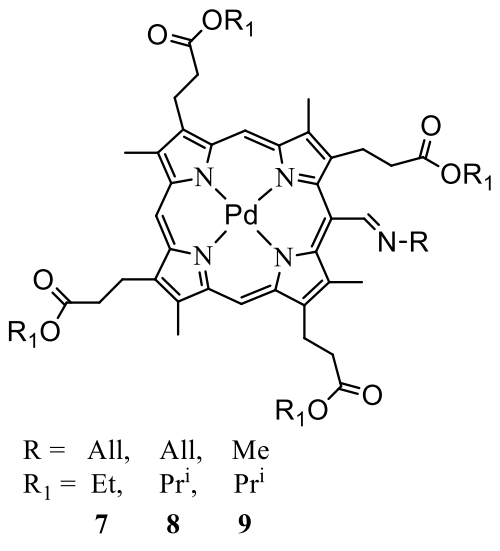

Figure 3. Azomethine derivatives of coproporphyrin I **7-9**.

Table 2. Maxima of the absorption bands in porphyrin electronic spectra (in $CHCl_3$).

Compound	Absorption bands, nm		
	Soret	Q_x	Q_y
1	391	511	545
2	391	482	608
3*a*	395	514	548
3*d*	396	514	549
3*b*,*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

Phosphorescence of the synthesized palladium complexes of azomethine derivatives was investigated in order to assess their potential photosensitizing ability. Figure 4 shows the photoluminescence spectrum of **3d** at 298 K. It has the fluorescence maximum at 555 nm with a minor peak at 605 nm and the strong phosphorescence maximum at 670 nm with minor peak at 728 nm. Low temperature phosphorescence of **9** recorded at 77 K has similar phosphorescence spectrum. (Fig. 5). Thus, the energy of the triplet states is significantly higher than that of the singlet $^1\Delta_g$ state of oxygen that allows for efficient energy transfer from the triplet PS to oxygen molecules. Palladium complexes of azomethine derivatives of mesochlorin e_6 (**4**,**5**) possess the longest absorption maximum of the Q_y band (616 nm) among the studied compounds. Accordingly, these compounds showed the phosphorescence maxima at the longest wavelengths – 810 and 822 nm respectively (Table 3). The lifetimes of the room temperature phosphorescence of the most compounds were rather similar: 71-84 μs. At 77 K the phosphorescence lifetime increased to 0.35-2 ms. It can be seen from data in table 3 that the peripherials 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 us 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.

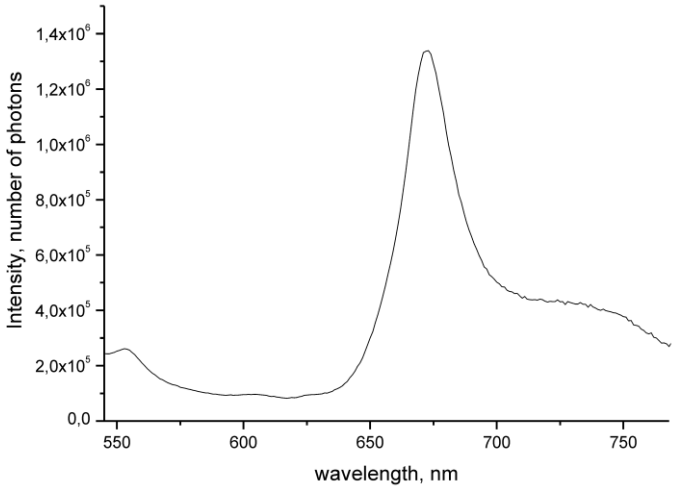

Figure 4. The photoluminescence (fluorescence and phosphorescence) spectrum of **3d** in polystyrene matrix at room tempearure. The main fluorescence band is seen at 555 nm, the main phosphorescence band – at 670 nm.

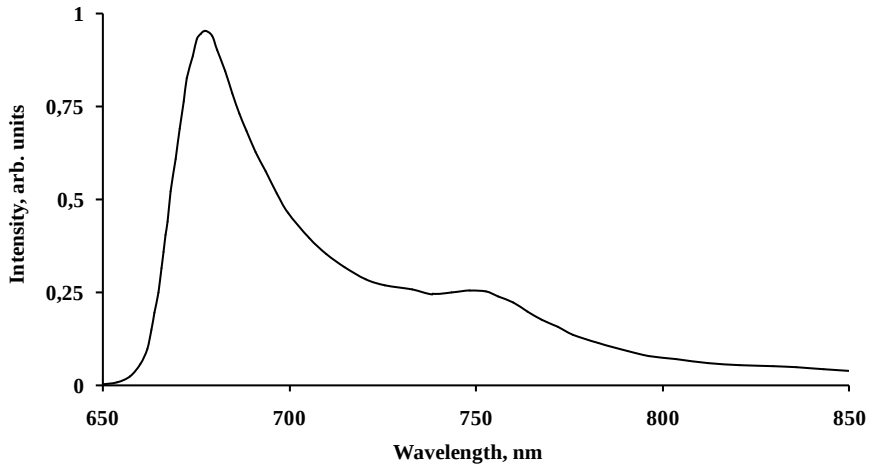

Figure 5. The phosphorescence spectrum of **9** in acetone at 77 K.

Table 3. Data of photophysical studies of porphyrins

Compound	Maxima of bands, nm		phosphorescence life time, μs	Quantum yield of singlet oxygen generation
	fluorescence	phosphorescence		
3a	552	671	84	-
3d	557	672	71	-
3bc	555	671	72	-
7	554	671	84	-
4	-	810	35	1,00 ± 0,07
5	-	822	80	1,00 ± 0,07
8	-	684	81	1,00 ± 0,05
9	-	678	65	1,00 ± 0,05

Quantum yield of generation of singlet oxygen was determined for compounds **4**, **5**, **8**, **9**. For this purpose we used a relative method using chemical trap 1,3-diphenylisobenzofuran (DPIBF). The standard compound was TPP (*meso*-tetraphenylporphin). Porphyrins were irradiated with light with a wavelength corresponding to the Q_y absorption band. Change of optical density was 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. Details of the calculation are given in Supporting information. Quantum yield of singlet oxygen generation was close to unity for all four investigated porphyrins (table 3). This result was quite expected, accounting for high yield, long lifetime and high energy of the triplet states of palladium complexes of porphyrins. Thus the investigated azomethine derivatives of porphyrins **3-5**, **7-9** can be promising precursors for development of the third generation PS for PDT. Further modification of these substances can be performed by transformation of the azomethine group in order to obtain 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 azomethine derivatives of porphyrins revealed that *meso*-methylimino and allylimino derivatives of mesochlorin e_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 at room temperature 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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Palladium Complexes of Azomethine Derivatives of Porphyrins as Potential Photosensitizers

V. S. Tyurin, D. R. Erzina, I. A. Zamilatskov, A.Yu. Chernyadiev, G. V. Ponomarev, D. V. Yashunskiy, A. V. Maksimova, K. V. Neverov, A. S. Kozlov, A. A. Krasnovsky, A. Yu. Tsivadze

Supporting Information

NMR spectra

At the ^{1}H NMR spectrum of compound **3*a*** (Fig. 1), the signals of three *meso*-H (9,91; 9,91; for 9.88) and one proton signal of the azomethine group (10,40) are at low field.

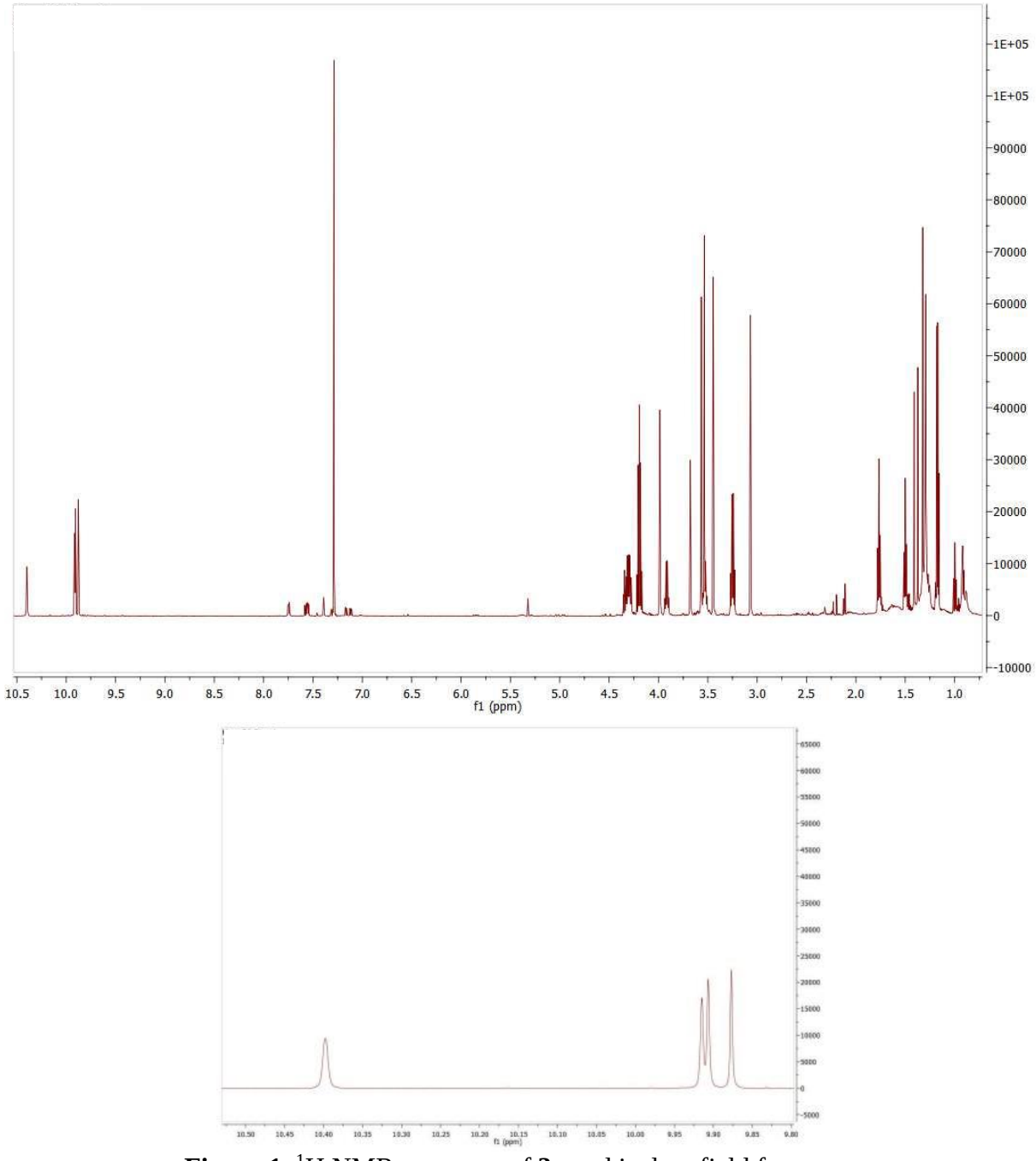


Figure 1. ^{1}H NMR spectrum of **3a** and its low field fragment.

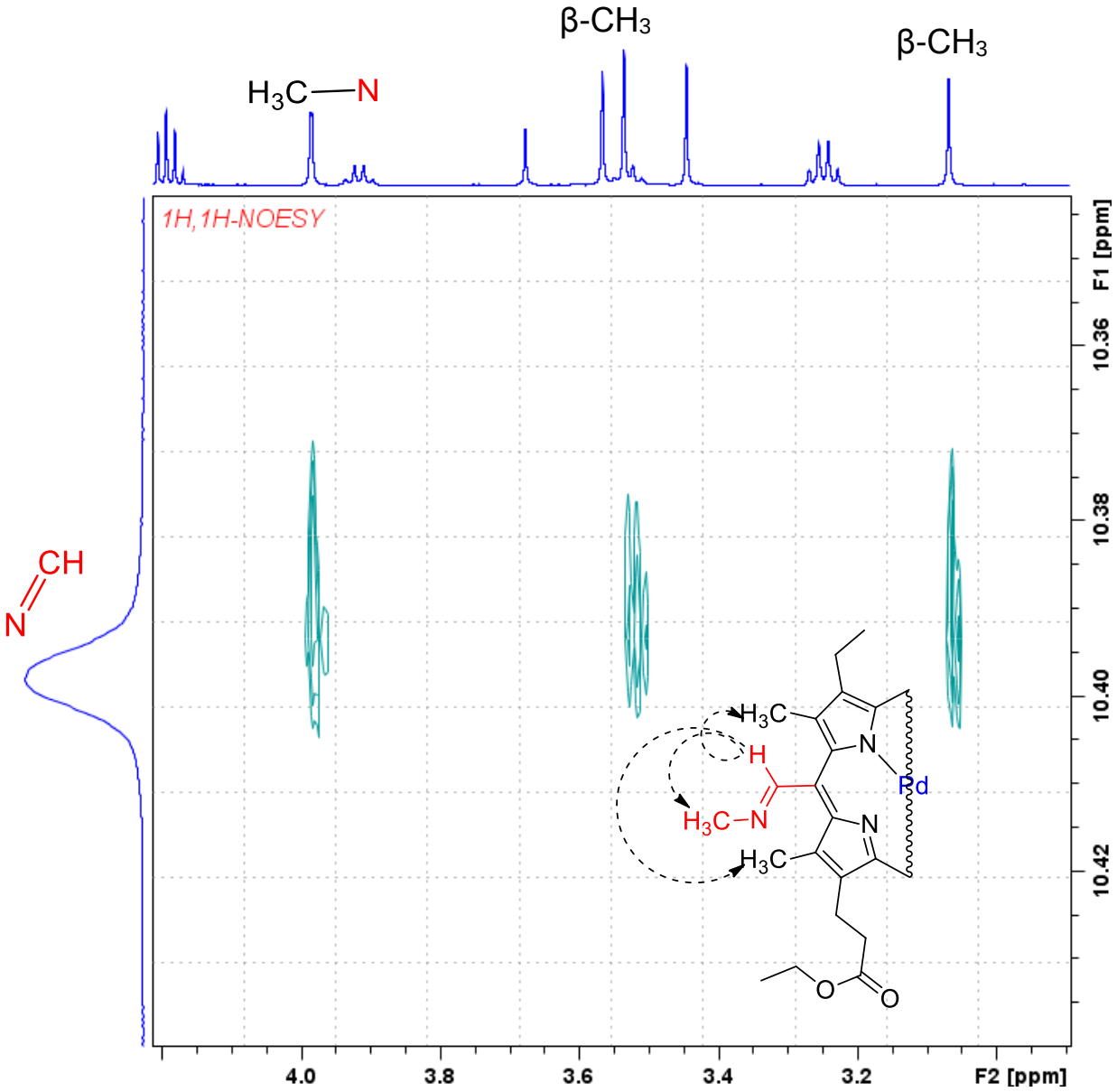


Figure 2. Fragment of NOESY spectrum of **3a**.

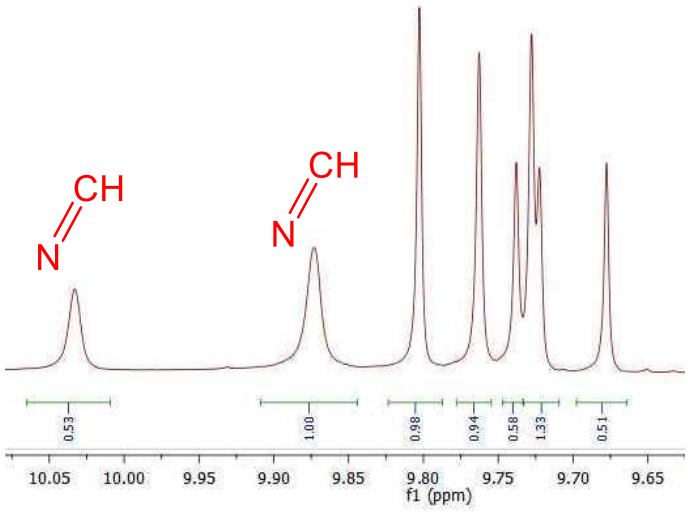


Figure 3. Fragment of ^{1}H NMR spectrum of isomers ***3b*** **+** ***3c***.

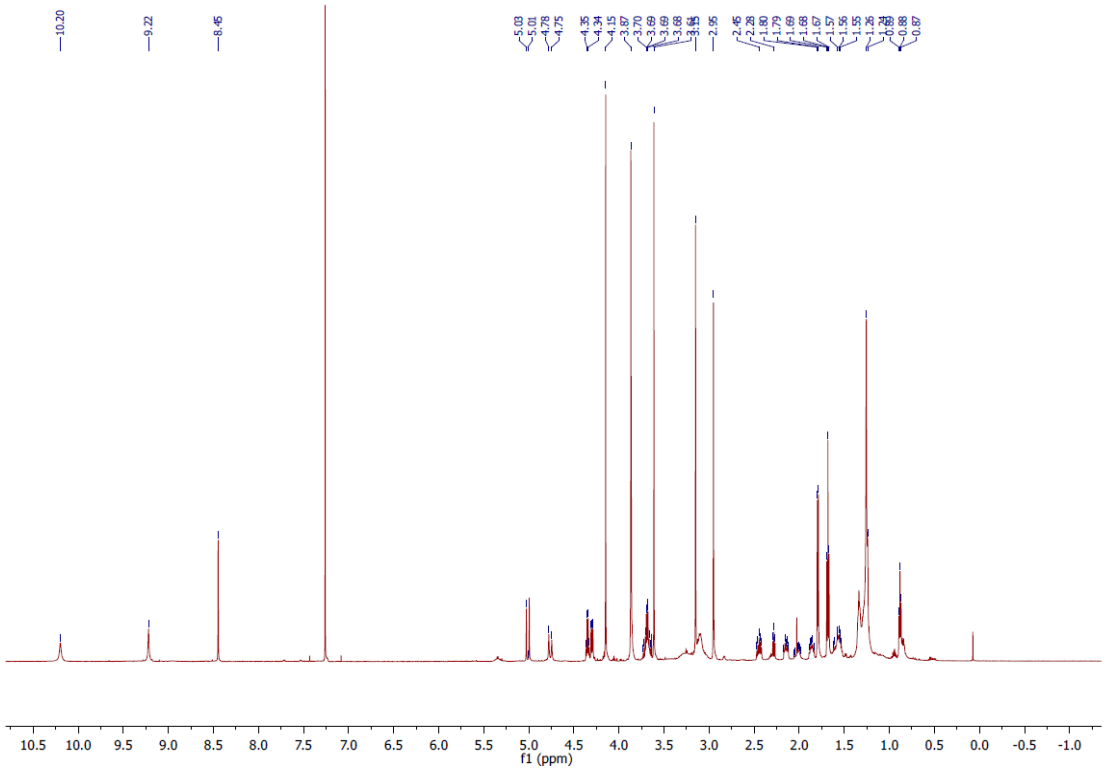


Figure 4. ^{1}H NMR spectrum of **5**.

Mass spectra

On the mass spectrum of the four isomers of ***3a-d*** two molecular ions are present, one corresponds to the Schiff base (M=768), and the other corresponds to cyclopentane derivative molecular ion (M=736) (Fig. 5).

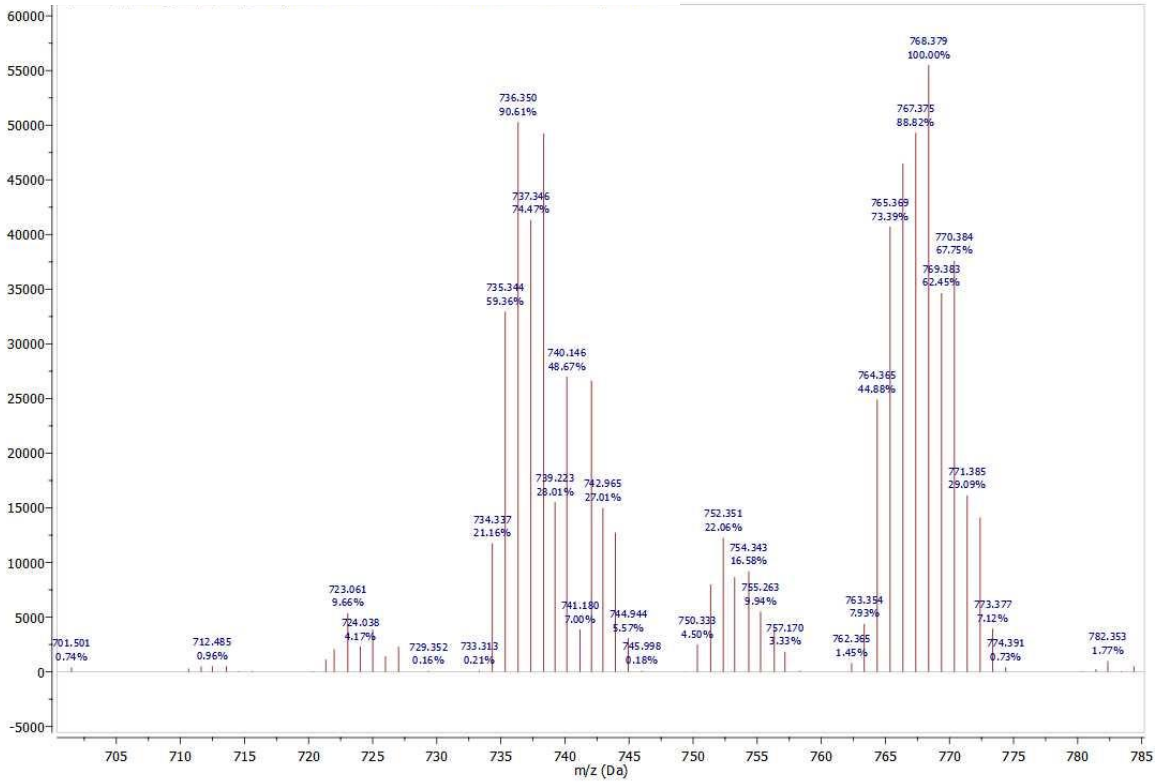


Figure 5. Fragment of the mass spectrum of isomers **3*a-d***.

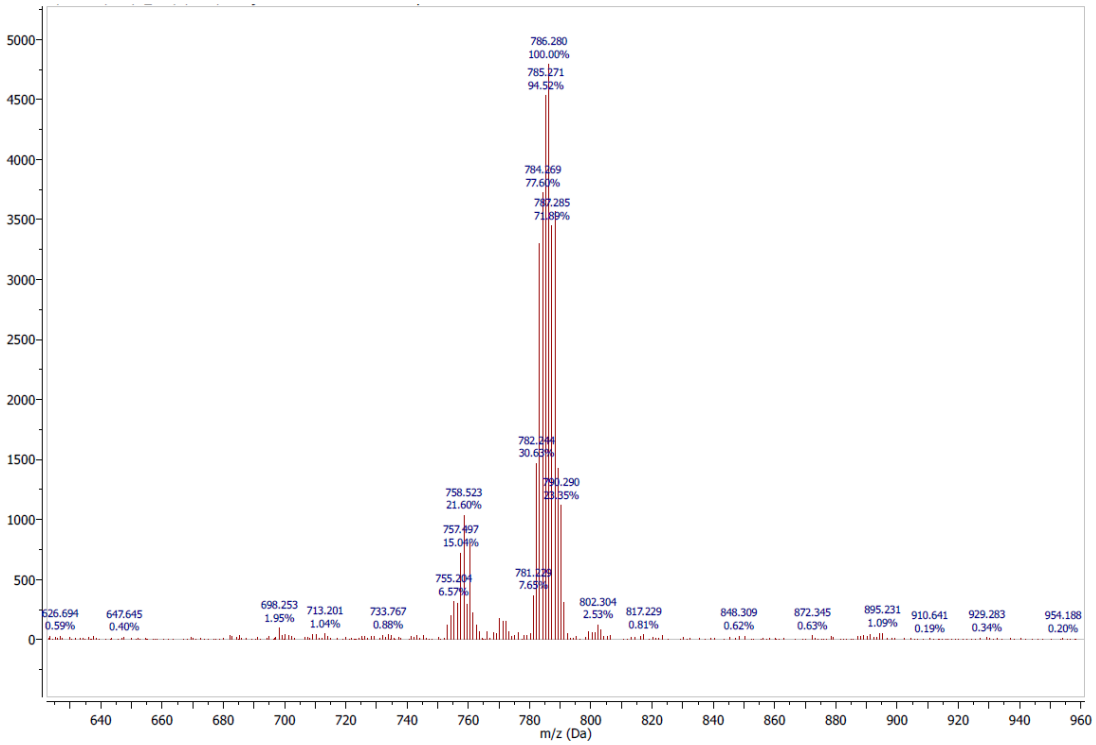


Figure 6. Fragment of the mass spectrum of **4**.

Electronic absorption spectra

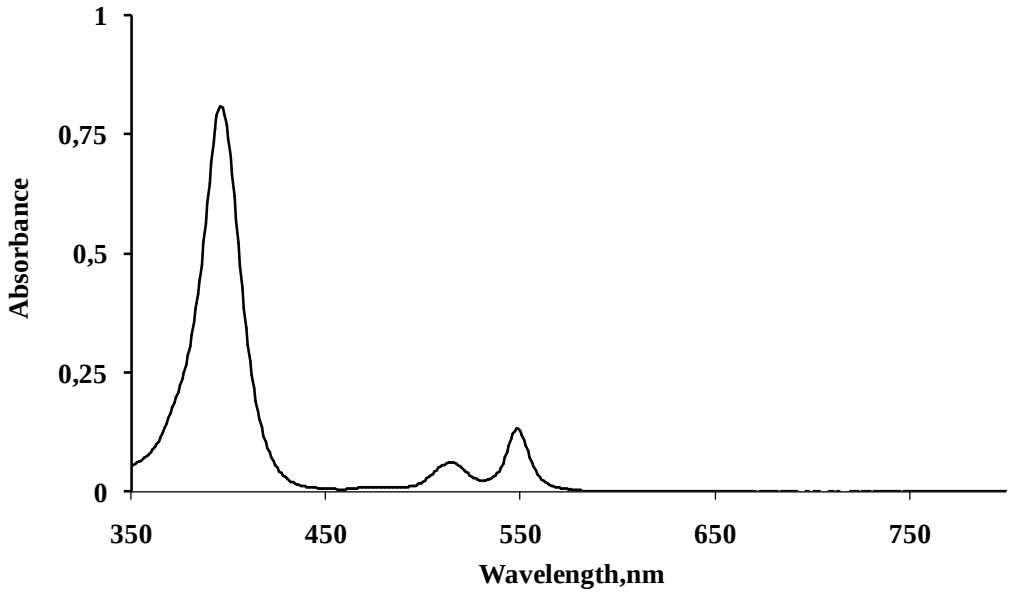


Figure 7. UV-Vis absorption spectrum of **8** in acetone.

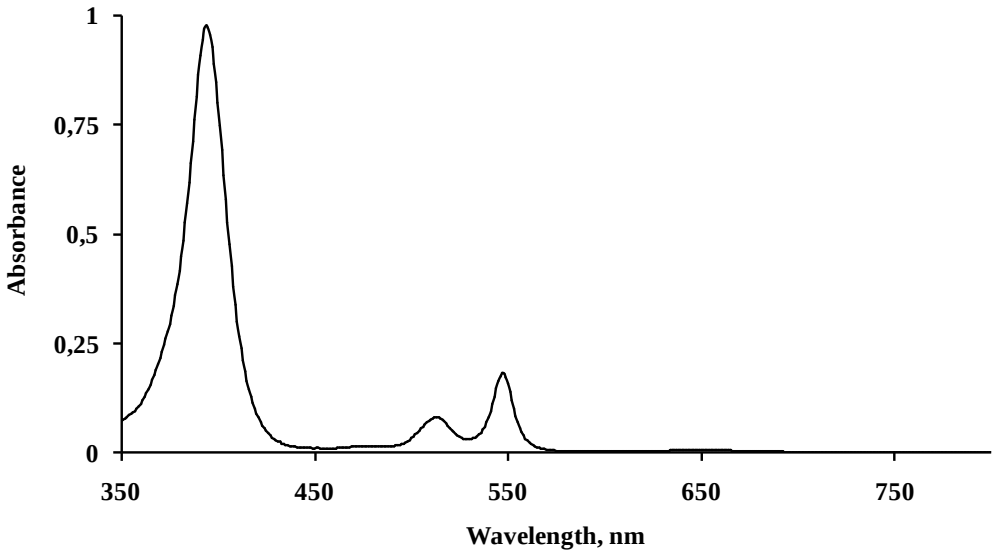


Figure 8. UV-Vis absorption spectrum of **9** in acetone.

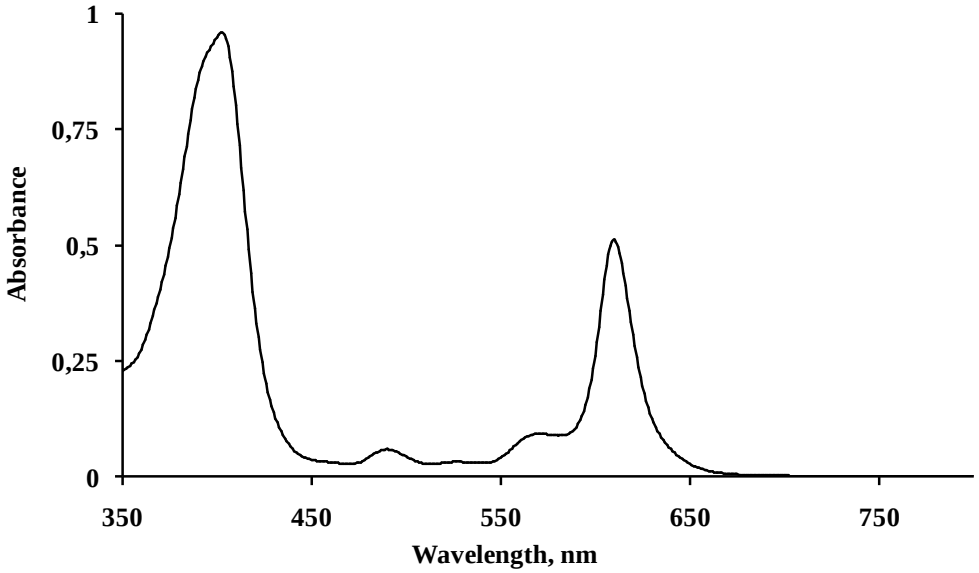


Figure 9. UV-Vis absorption spectrum of **4** in acetone.

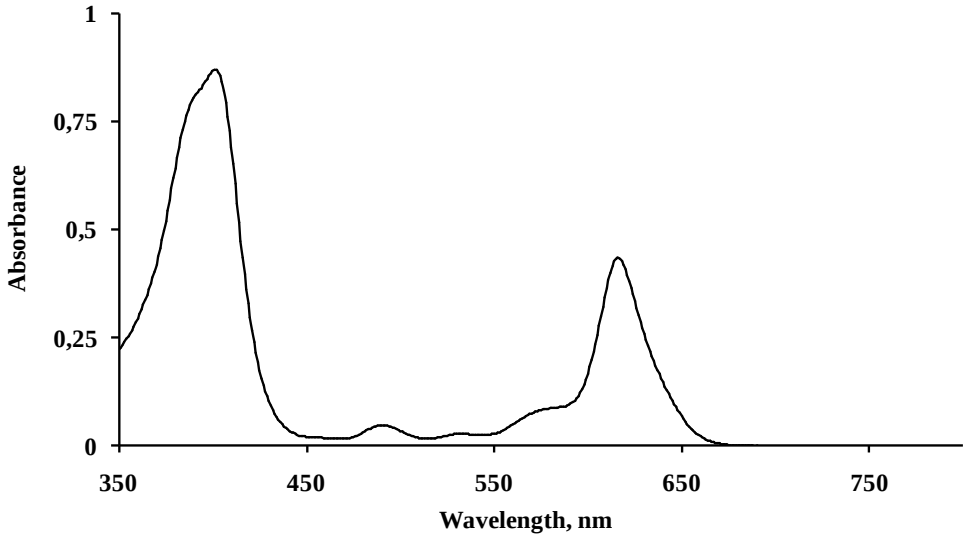


Figure 10. UV-Vis absorption spectrum of **5** in acetone.

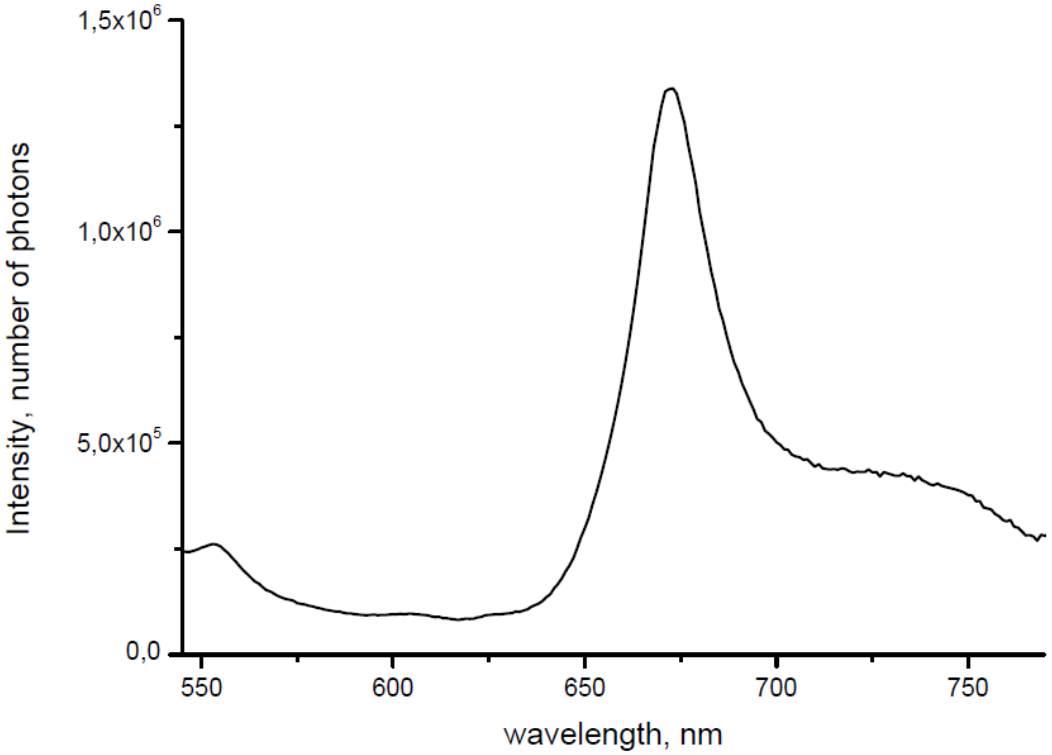


Figure 11. Photoluminescence spectrum of **3*a*** in polystyrene film. Excitation at 397 nm.

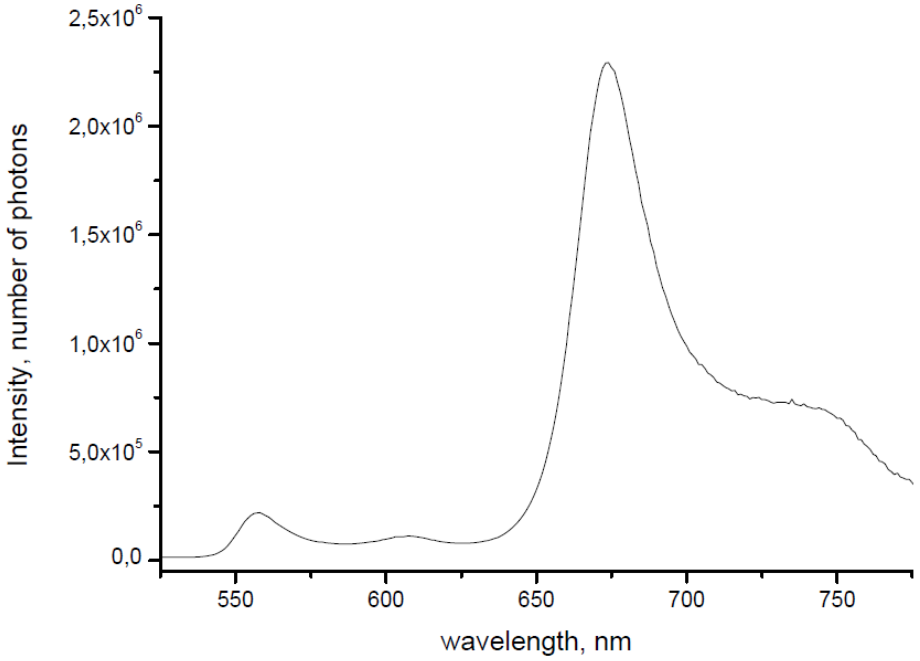


Figure 12. Photoluminescence spectrum of **3*b*+*3c*** in polystyrene film. Excitation at 397 nm.

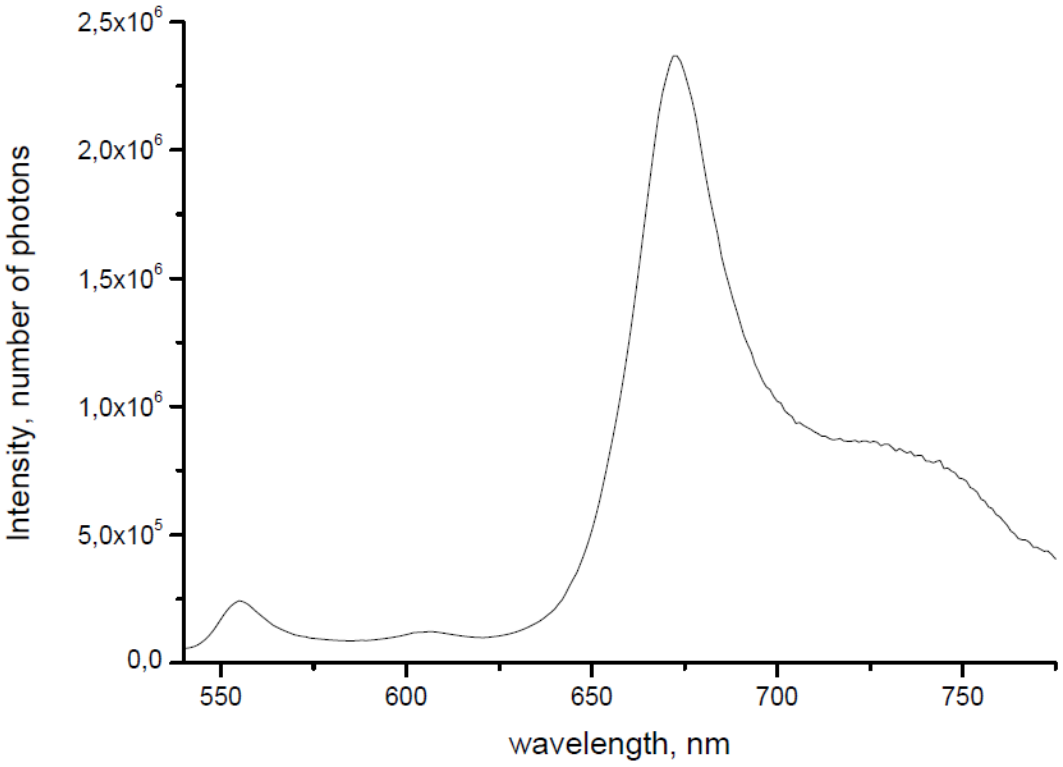


Figure 13. Photoluminescence spectrum of **3*d*** in polystyrene film. Excitation at 397 nm.

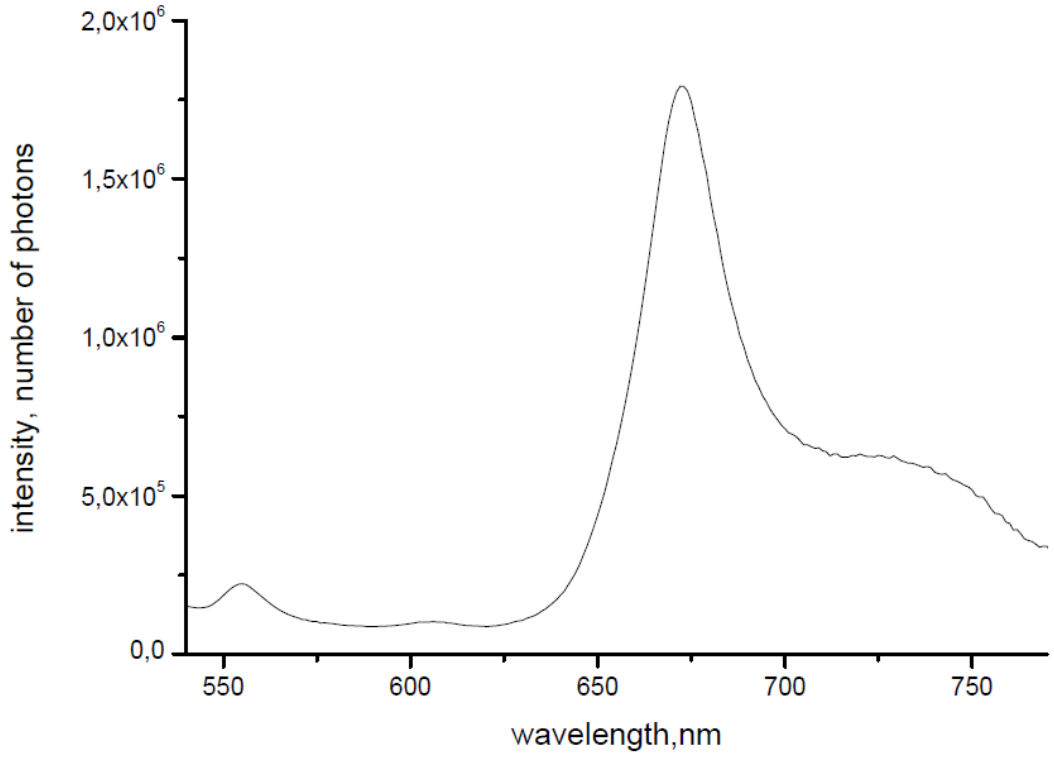


Figure 14. Photoluminescence spectrum of **7** in polystyrene film. Excitation at 397 nm.

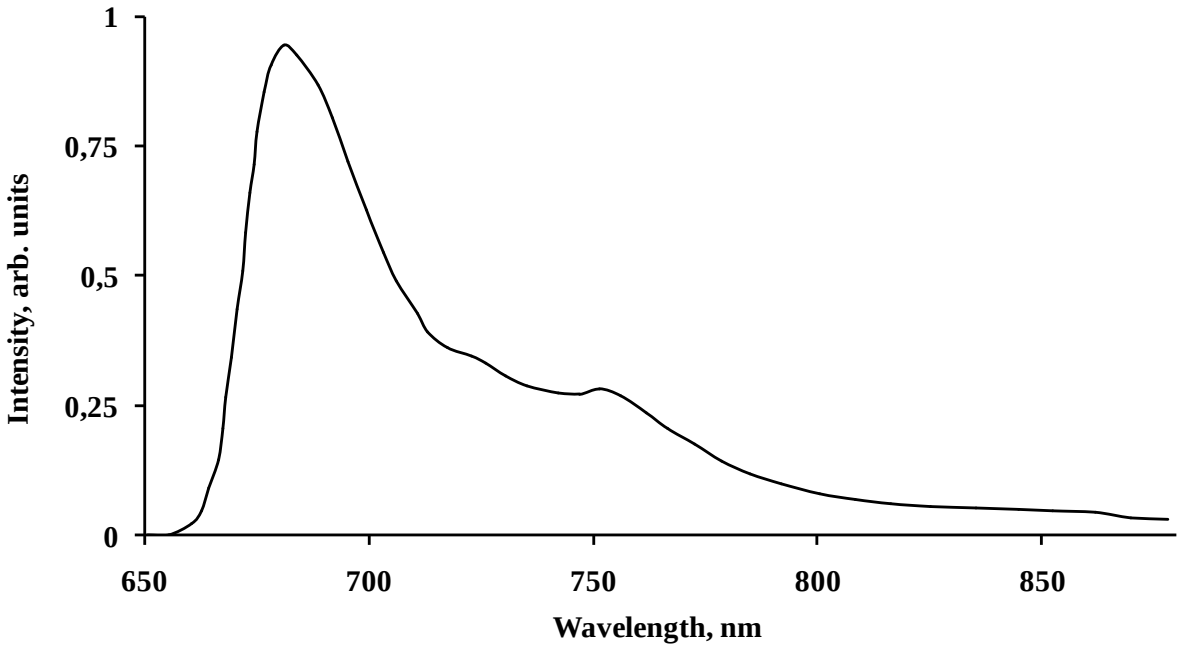


Figure 15. The phosphorescence spectrum of **8** in acetone at 77 K.

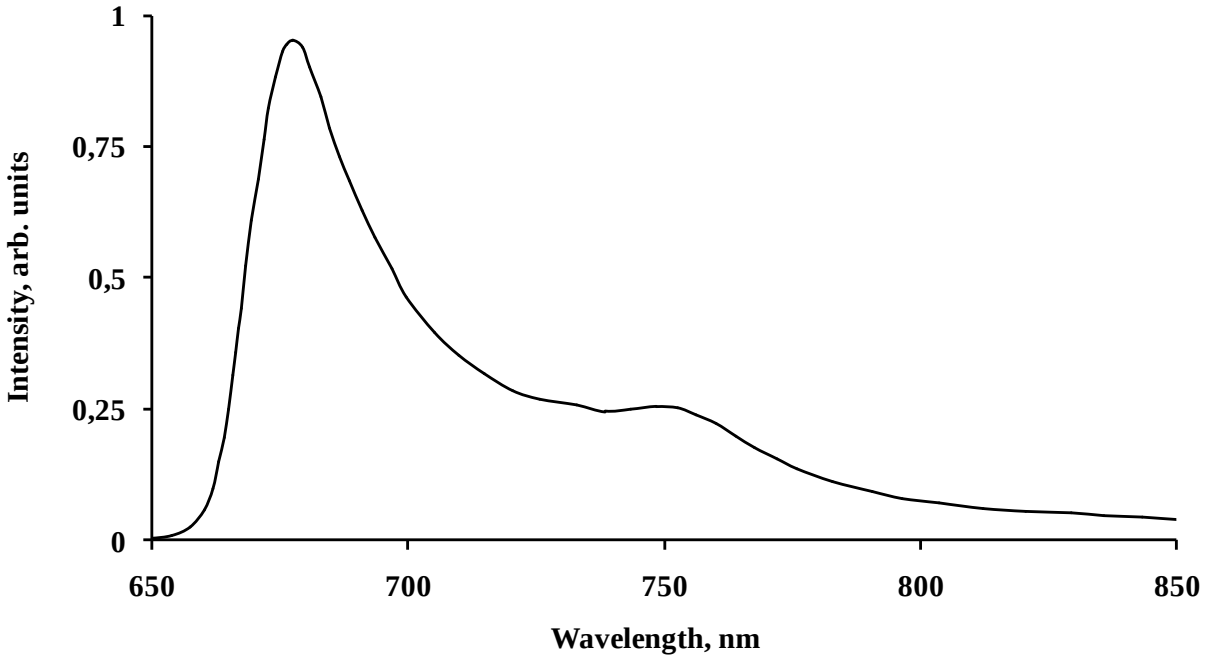


Figure 16. The phosphorescence spectrum of **9** in acetone at 77 K.

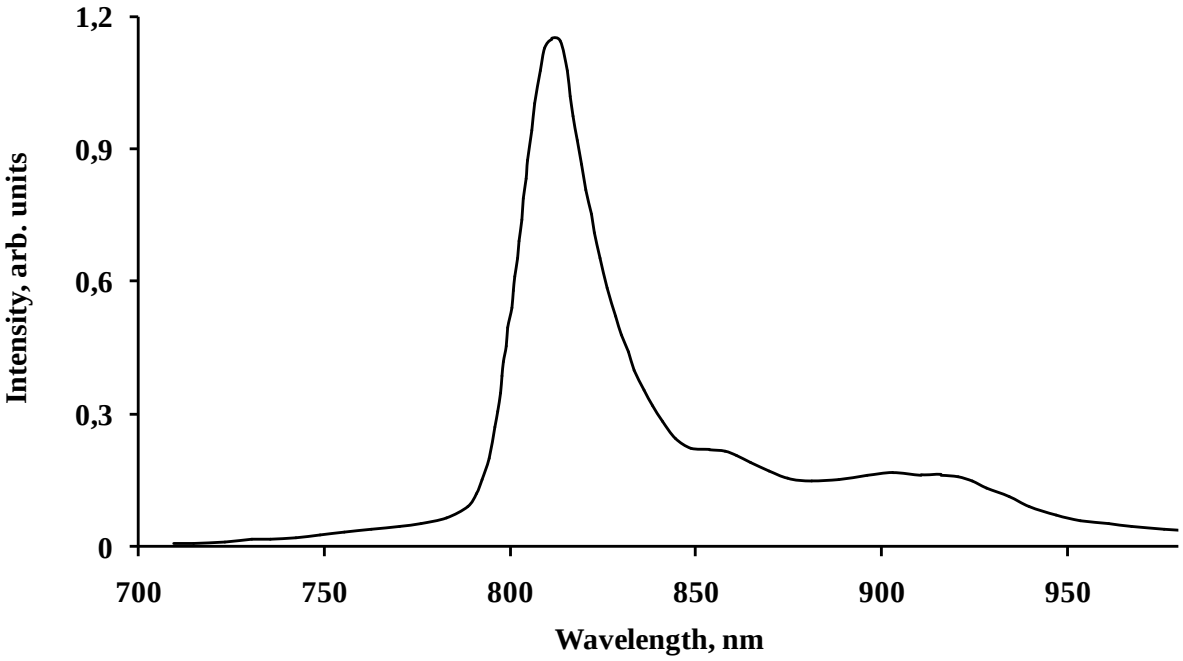


Figure 17. The phosphorescence spectrum of **4** in acetone at 77 K.

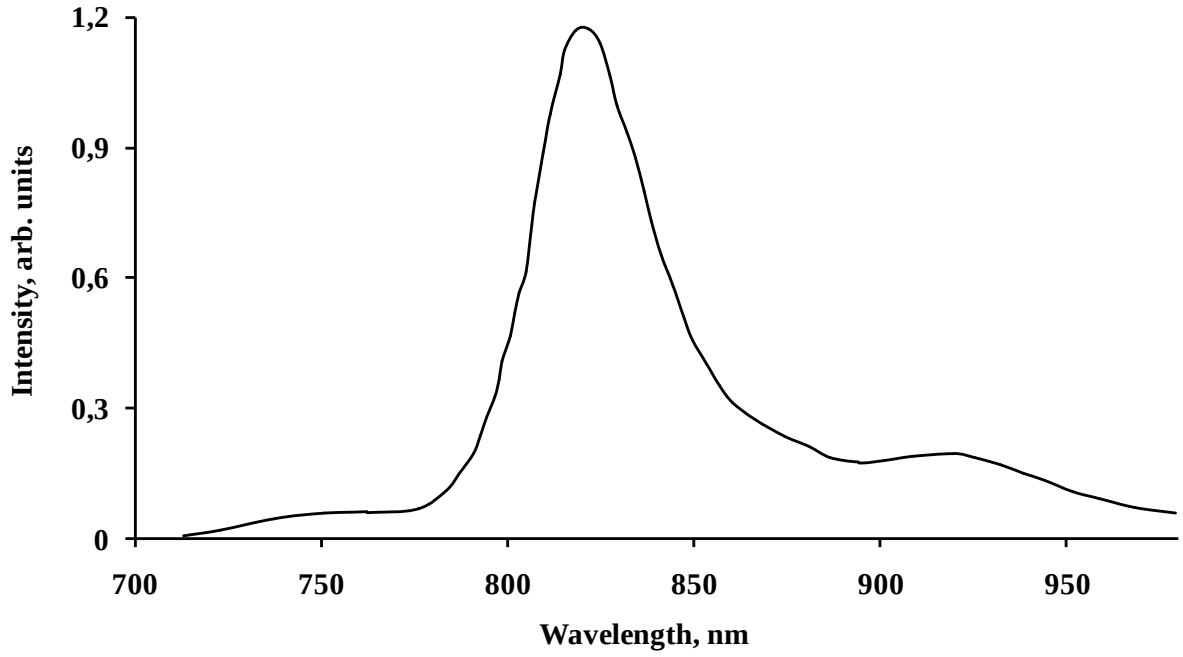


Figure 18. The phosphorescence spectrum of **5** in acetone at 77 K.

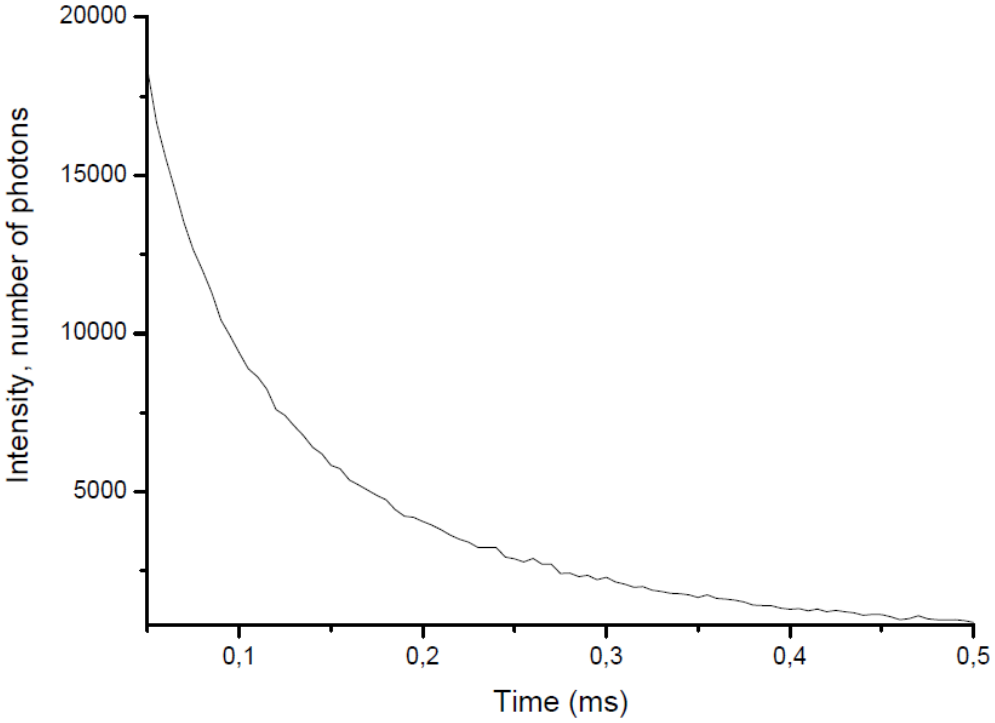


Figure 19. The phosphorescence decay curve for **3*a*** in polystyrene film at room temperature. Excitation at 397 nm, emission at 670 nm.

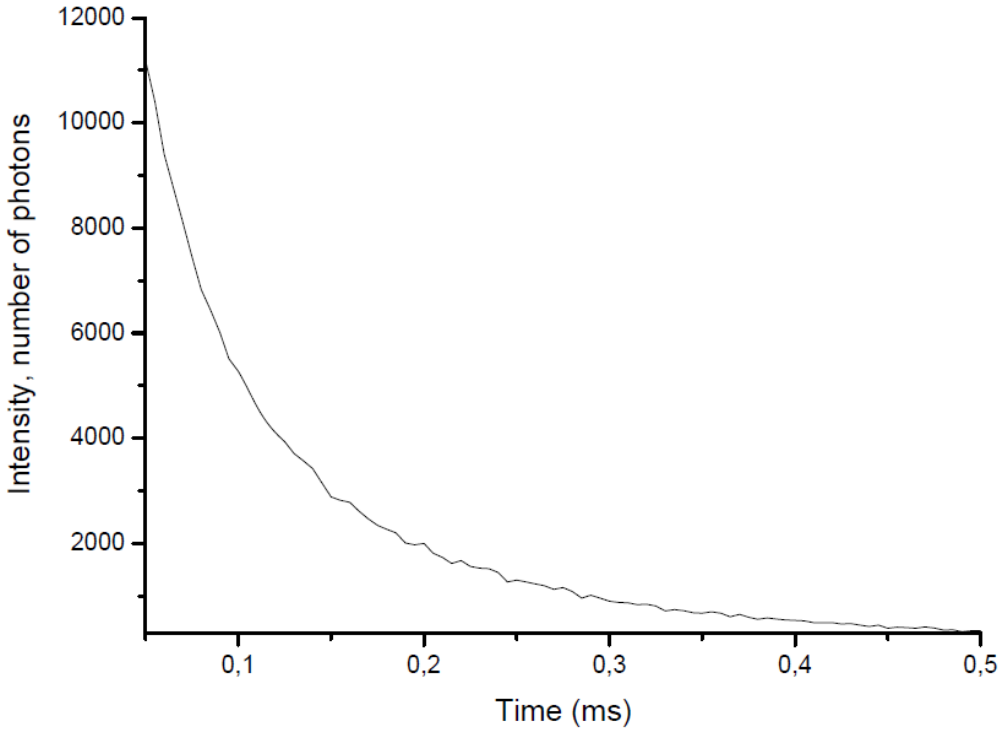


Figure 20. The phosphorescence decay curve for **3*b*+3*c*** in polystyrene film at room temperature. Excitation at 397 nm, emission at 670 nm.

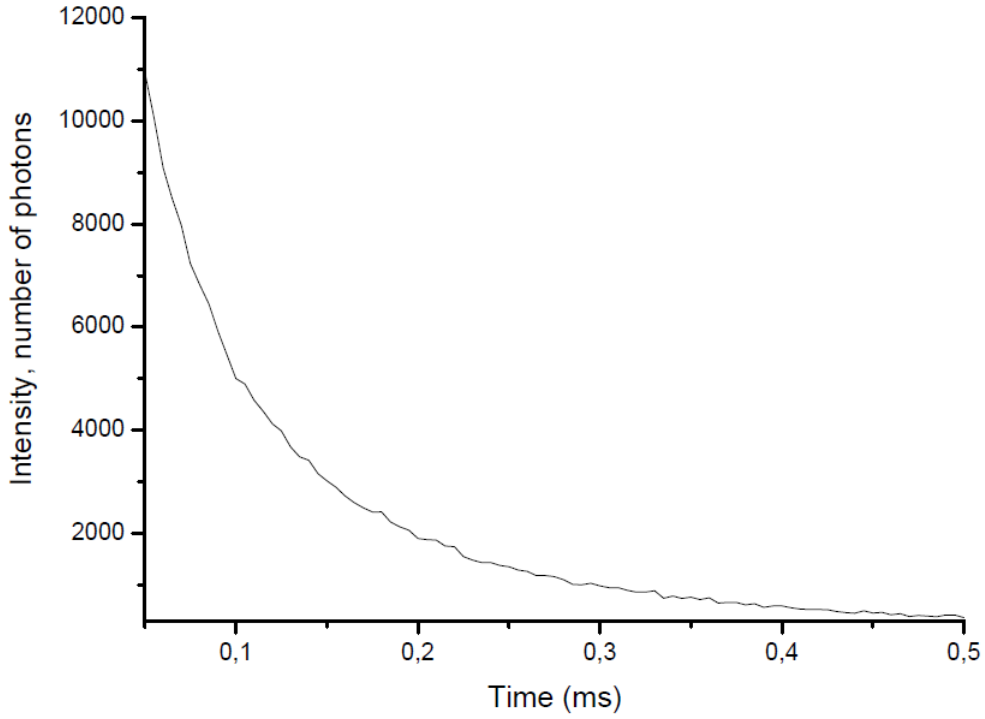


Figure 21. The phosphorescence decay curve for **3*d*** in polystyrene film at room temperature. Excitation at 397 nm, emission at 670 nm.

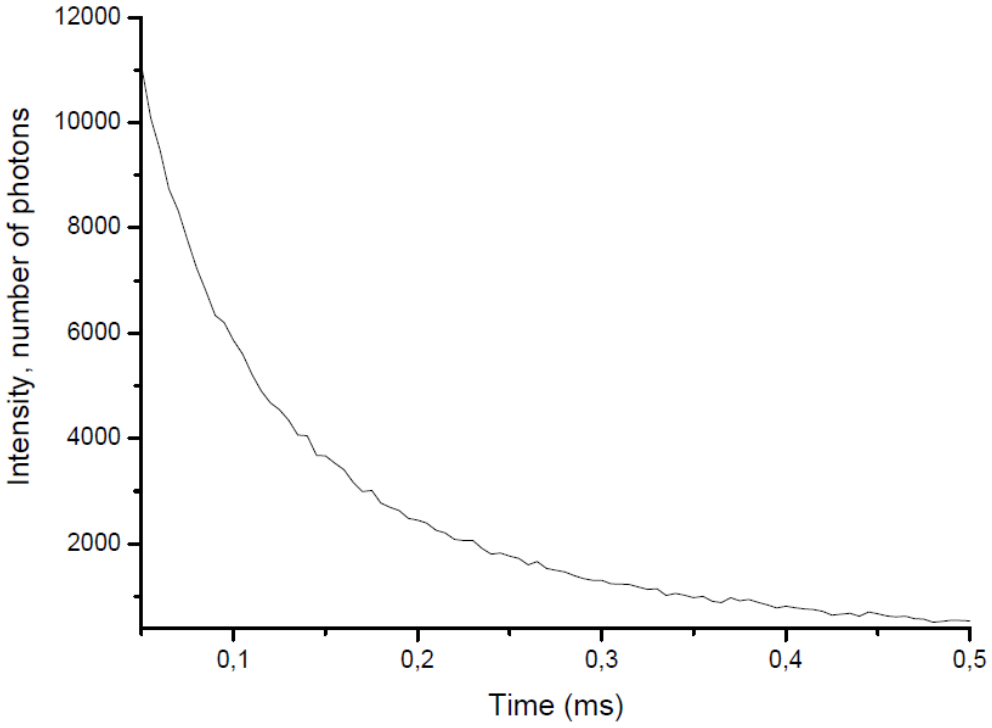
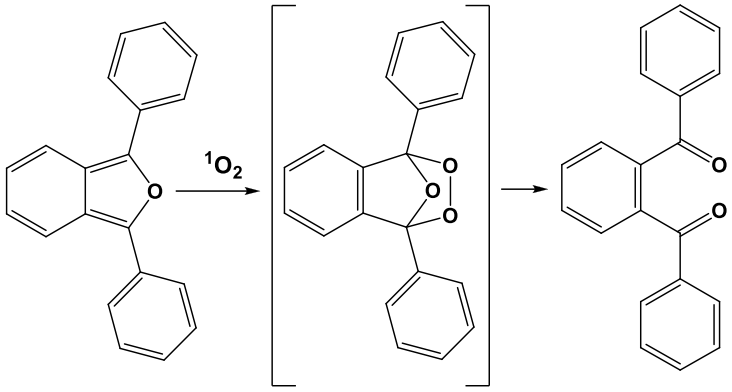


Figure 22. The phosphorescence decay curve for **7** in polystyrene film at room temperature. Excitation at 397 nm, emission at 670 nm.

The calculation of the quantum yield of singlet oxygen generation

The quantum yield of singlet oxygen generation by the studied compounds was determined by the relative method using chemical traps. The standard compound was TPP (*meso*-tetraphenylporphin). The quantum yield of singlet oxygen generation by TPP in acetone is known to be 0.65 ± 0.05 [1,2]. 1,3-Diphenylisobenzofuran (DPIBF) was used as a chemical trap. The interaction of this trap with singlet oxygen is a result of purely chemical process indicated on the scheme below [3]. The products of the reaction (mainly 1,2-dibenzoylbenzene) have no absorption bands in the visible spectral region. Therefore, the amount of singlet oxygen formed during the photoreaction can be measured via the loss of optical density in the absorption maximum of DPIBF (412 nm).



For the determination of the singlet oxygen quantum yields, solutions of tested compounds and DPIBF in a fixed volume of solvent were prepared. Measurements were performed in a square quartz fluorescence cuvettes with optical path of 1 cm. The solutions were irradiated with the monochromatic light from the xenon lamp containing excitation unit from Perkin Elmer MPF-44B fluorimeter. Light intensity was measured using a ThorLabs unit PM-100D with the sensor head S120VC. Wavelengths of the excitation light corresponded to the absorption maxima of tested compounds, 550 or 615 nm. The monochromator slit width corresponded to 5 nm. Under irradiation, change of optical density was controlled at the absorption maximum of DPIBF and at the poprphyrin absorption band corresponding to the wavelength of excitation. Intensity of irradiation was set so that photobleaching of the sensitizer was not detected during irradiation time. The optical density was determined using a two-beam spectrophotometer Hitachi U-3400. DPIBF concentration corresponded to optical density equal to 0.80-1.10 in the DPIBF absorption maximum. As seen from Fig. 23-26 the overall optical density of samples in this maximum was much greater due to contribution of the Soret band of tested porphyrins.

The quantum yields were calculated from comparison of the rates of DPIBF bleaching in solutions of the tested compounds and TPP. Each measurement was repeated 3-4 times. The calculation was performed according to the following formula:

$$\varphi_{\Delta} = \varphi_{\Delta St} \cdot \frac{\Delta D_{Cmp} \cdot t_{irrSt} \cdot I_{St} \cdot (1-10^{-D_{St}}) \cdot \lambda_{St}}{\Delta D_{St} \cdot t_{irrCmp} \cdot I_{Cmp} \cdot (1-10^{-D_{Cmp}}) \cdot \lambda_{Cmp}},$$

where $\varphi_{\Delta St}$ is the quantum yield of singlet oxygen generation by standard compound; ΔD_{Cmp} is the average rate of DPIBF bleaching in solutions of tested compounds, ΔD_{St} is the average rate of DPIBF bleaching in solutions of TPP, t_{irrCmp} is the duration of light exposure of the mixture of

DPIBF with tested compounds in sec, t_{irrSt} is the duration of light exposure of the mixture of DPIBF with TPP in sec, I_{Cmp} and I_{St} are intensities of light flux from the xenon lamp in the experiments with tested compounds and TPP in μW, D_{Cmp} and D_{St} are optical densities of tested compounds and TPP corresponding to the wavelengths of the exciting light λ_{Cmp} and λ_{St} (in nm).

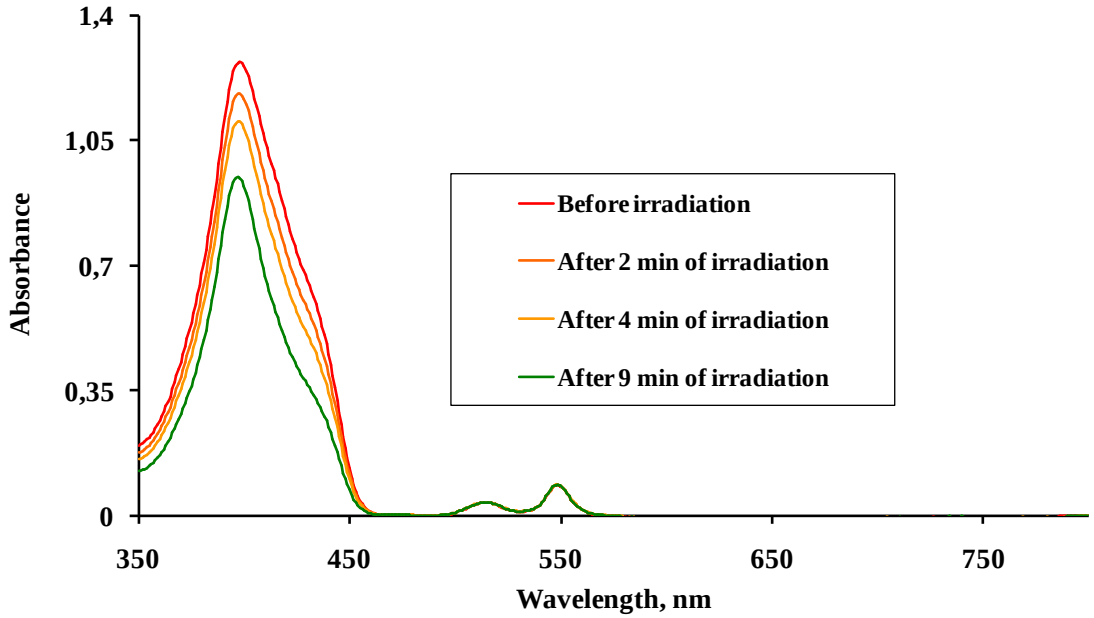


Figure 23. Absorption spectrum of mixture of **8** and DPIBF in acetone before and after irradiation at 550 nm (in the presence of oxygen).

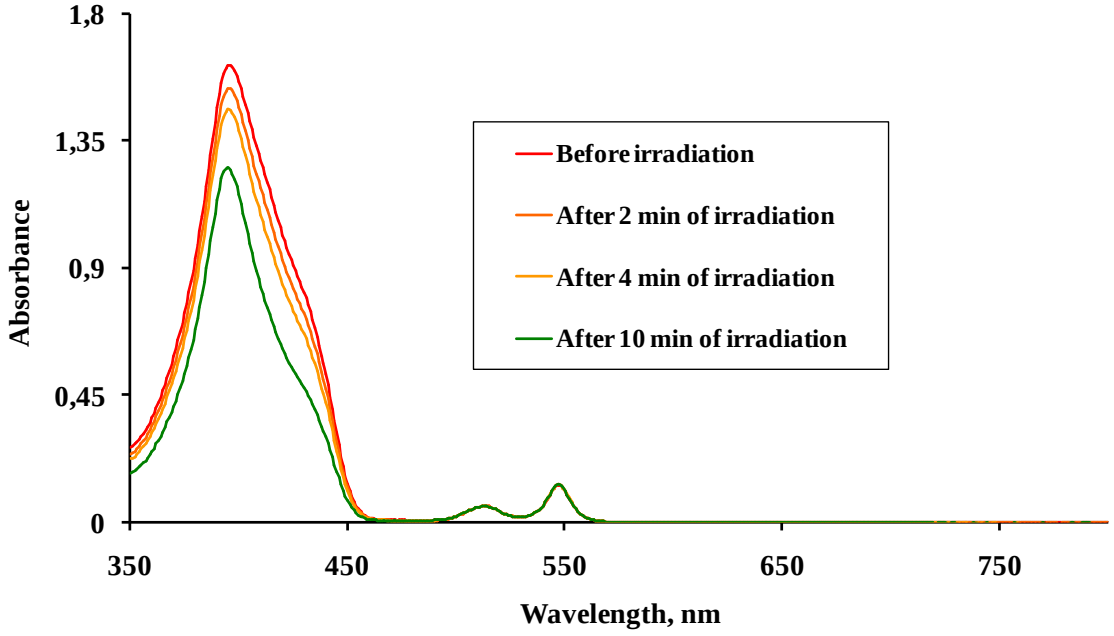


Figure 24. Absorption spectrum of mixture of **9** and DPIBF in acetone before and after irradiation at 550 nm (in the presence of oxygen).

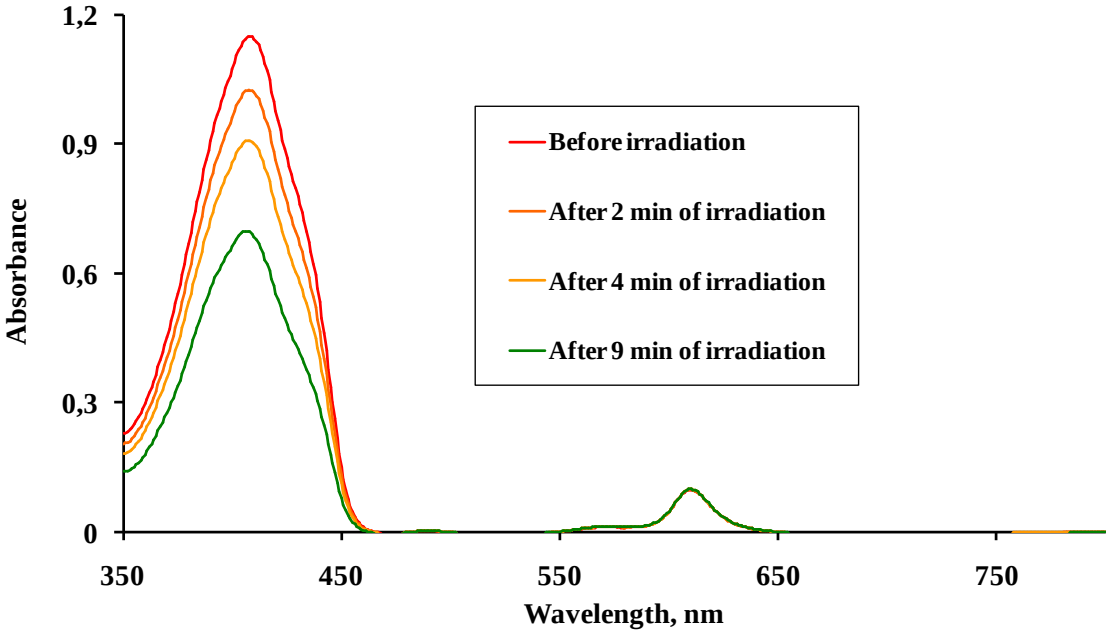


Figure 25. Absorption spectrum of mixture of **4** and DPIBF in acetone before and after irradiation at 615 nm (in the presence of oxygen).

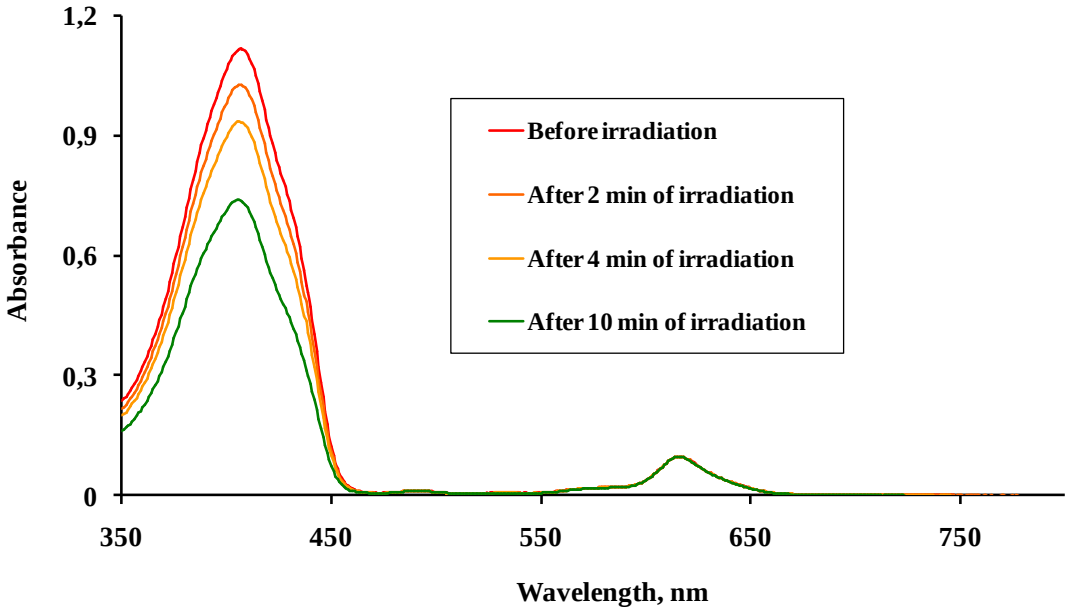


Figure 26. Absorption spectrum of mixture of **5** and DPIBF in acetone before and after irradiation at 615 nm (in the presence of oxygen).

Table 1. Experimental data for calculation of the quantum yield of singlet oxygen generation.

#	Experiment	λ, nm	I, μW	t_{irr}, sec	ΔD*	D_{Cmp} or D_{St}*	φ_{Δ}
1	**8** + DPIBF	550	94	120	0.136	0.115	1.00 ± 0.07
	TPP+ DPIBF	512	105	600	0.112	0.026	0.65 ± 0.05
2	**9** + DPIBF	545	97	120	0.140	0.114	1.00 ± 0.05
	TPP+ DPIBF	512	105	600	0.112	0.026	0.65 ± 0.05
3	**5** + DPIBF	615	107	120	0.176	0.116	1.00 ± 0.05
	TPP+ DPIBF	512	105	600	0.112	0.026	0.65 ± 0.05
4	**4** + DPIBF	613	98	120	0.180	0.134	1.00 ± 0.07
	TPP+ DPIBF	512	105	600	0.113	0.026	0.65 ± 0.05

* Average values for several experiments.

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2) F. Wilkinson, W. P. Helman and A. B. Ross. Quantum yields of the photosensitized formation of the lowest electronically excited singlet states of molecular oxygen. // *J. Phys. Chem. Ref. Data*. 1993, vol. 22, pp. 113-262.

3) R. H. Young, D. Brewer and R. A. Keller. The determination of rate constants of reaction and lifetimes of singlet oxygen in solution by a flash photolysis technique. // *J. Am. Chem. Soc.* 1973, vol. 95, pp. 375-379.