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The synthesis of new potential photosensitizers [1]. Part 2. Tetrakis-(hydroxyphenyl)porphyrins with long alkyl chain in the molecule

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ABSTRACT

Several new derivatives of tetrakis(hydroxyphenyl)porphyrin were synthesized and their physicochemical data were established. These data were further assessed in terms of the synthesized compounds' usefulness as potential photosensitizers in anticancer photodynamic therapy. Absorption and fluorescence spectra, as well as triplet state lifetime were determined along with the compounds' stability and capacity to generate singlet oxygen. They obtained were compared to the corresponding data pertaining to a well-known and clinically admitted photosensitizing drug (Foscan).

1. Introduction

PDT usefulness in anticancer treatment has been so far limited. Undoubtedly, one of the reasons has been a small number of photosensitizers admitted for clinical use and capable of generating singlet oxygen which would rapidly kill cancer cells in various body locations [2]. Another problem is the delivery of photosensitizer to the target site and availability of suitable methods is critically important. The simplest approach is direct intratumoral injection of such compound but this is not always feasible. Along with search for novel photosensitizers also adequate methods of their transport in the living organism are sought. Among known methods liposome encapsulation has been used. Appropriate composition of liposomes, besides assuring targeted delivery, can also assure resistance of the transported photosensitizer against body defense mechanisms. The drug (photosensitizer) can be liposome-encapsulated and delivered intracellularly or it can be a part of liposomal lipid bilayer which would ultimately lead to its integration with cellular membrane. Destruction of cells follows their irradiation and can occur both intracellularly and involving cell membrane.

We based our search for novel photosensitizers on the structure of Foscan which is a chlorine obtained on the basis of tetrakis(3-hydroxyphenyl)-porphyrin. In his study, Bonnett determined biological activity of this compound and other similar hydroxyl derivatives of tetrakisphenylporphyrin (especially para isomers). The results were comparable to those of Photofrin, the first porphyrin derivative admitted for clinical use. Tetrakis-(3-hydroxyphenyl) porphyrin (**1**) turned out to be 25–30 times more active than the earlier-studied HpD [3,4]. It did not, however, find use in PDT photosensitizer. Two reduced derivatives of this porphyrin, namely chlorin (**2**) and bacteriochlorin (**3**) proved to be better photosensitizers owing to stronger absorption in the red part of visible light spectrum compared to the starting porphyrin [5–8] (Fig. 1)

Since our goal was to obtain nontoxic porphyrin that could be transported by liposomes we had to pay particular attention to its hydrophobic–hydrophylic properties. The manner in which porphyrin is anchored in liposomes is of importance [8]. Literature data suggest that hydrophilic porphyrins linked to long hydrophobic chains are incorporated much easier into micelle formed by fatty substances. This process, and location of porphyrin in particular, is dependent on pH of the environment in which micelle are formed and on porphyrin concentration [9–11]. These factors also affect another feature, unfavorable from the perspective of PDT

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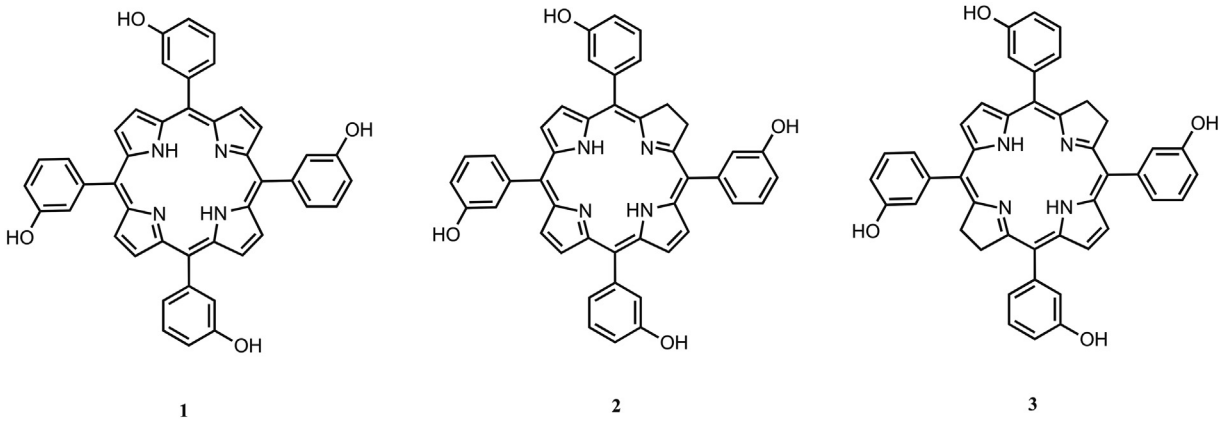


Fig. 1. Structures of **1**, **2**, **3**.

application, namely porphyrin aggregation. Several aqueous porphyrin solutions are prone to aggregation which causes their precipitation from such solutions.

Using porphyrin **1** as a *leading structure*, we decided to modify it in such a manner so that its derivatives were distinctly amphiphilic. This feature allows porphyrin molecules to partially dissolve in less polar liquids which can be of use in micellar or liposomal transport. *Vesicle* formation has been observed for certain amphiphilic porphyrins which is not favorable for their incorporation into lipid layers [12]. This phenomenon took place for porphyrins with three pirydyl substituents. Encapsulating a very polar porphyrin molecule inside a liposome is also possible albeit much less probable than using a long amphiphilic fragment (such as porphyrin linked to long alkyl chain) to co-form micelle or liposomes.

In line with our expectations, the best suited compound to interact with lipid components of micelles or liposomes would be porphyrin **4** with three hydroxyl groups in *meta* position and long alkyl chain in *para* position (this is shown schematically in Fig. 2). Alternative attachment of alkyl chain (in *meta* or *orto* position) might cause spatial hindrance during porphyrin incorporation into semipermeable membranes of micelles or liposomes. However, micellar incorporation of porphyrin with long alkyl chain attached in position other than *para* position of the side phenyl ring cannot be ruled out completely. Accordingly, we aimed at preparing porphyrins with less "favorable" positioning of alkyl chain. The synthesized and characterized compounds are shown in Fig. 3 and Table 1. They include alkylporphyrins with ether bonds between alkyl and phenyl moieties (the least favorable kind of bond from biological perspective), as well as ester and amid derivatives. Another interesting group of porphyrins are those with methoxy substituents. The latter, placed in the vicinity of hydroxyl groups, decrease polarity of this fragment of the molecule which translates into decisive effect upon hydrophobic–hydrophylic properties of the whole molecule. Last but not least is the length of the alkyl fragment attached to porphyrin. Our earlier experience has shown that optimum length of this fragment is that corresponding to 16 carbon atoms in the chain [13]. This is why we used in the synthesis cetyl (hexadecyl) radicals or palmitic acid fragments. The obtained compounds characterized in this study are based on tetrahydroxyl porphyrin **1**, and on several other starting porphyrins shown below (**5**–**7**).

These compounds were obtained in one-stage direct synthesis starting from appropriate hydroxybenzaldehydes. Earlier reports describe synthesis of these compounds in two stages. First, suitable methoxy or acetyloxyphenylporphyrins are obtained and then protecting groups are removed [4].

The most relevant photophysical data are presented in Tables 2–4.

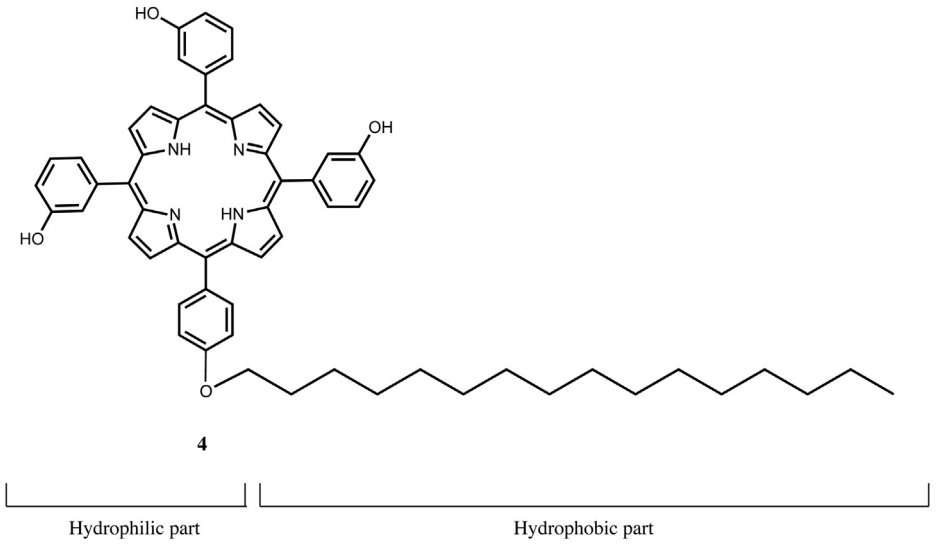


Fig. 2. Structure of **4**.

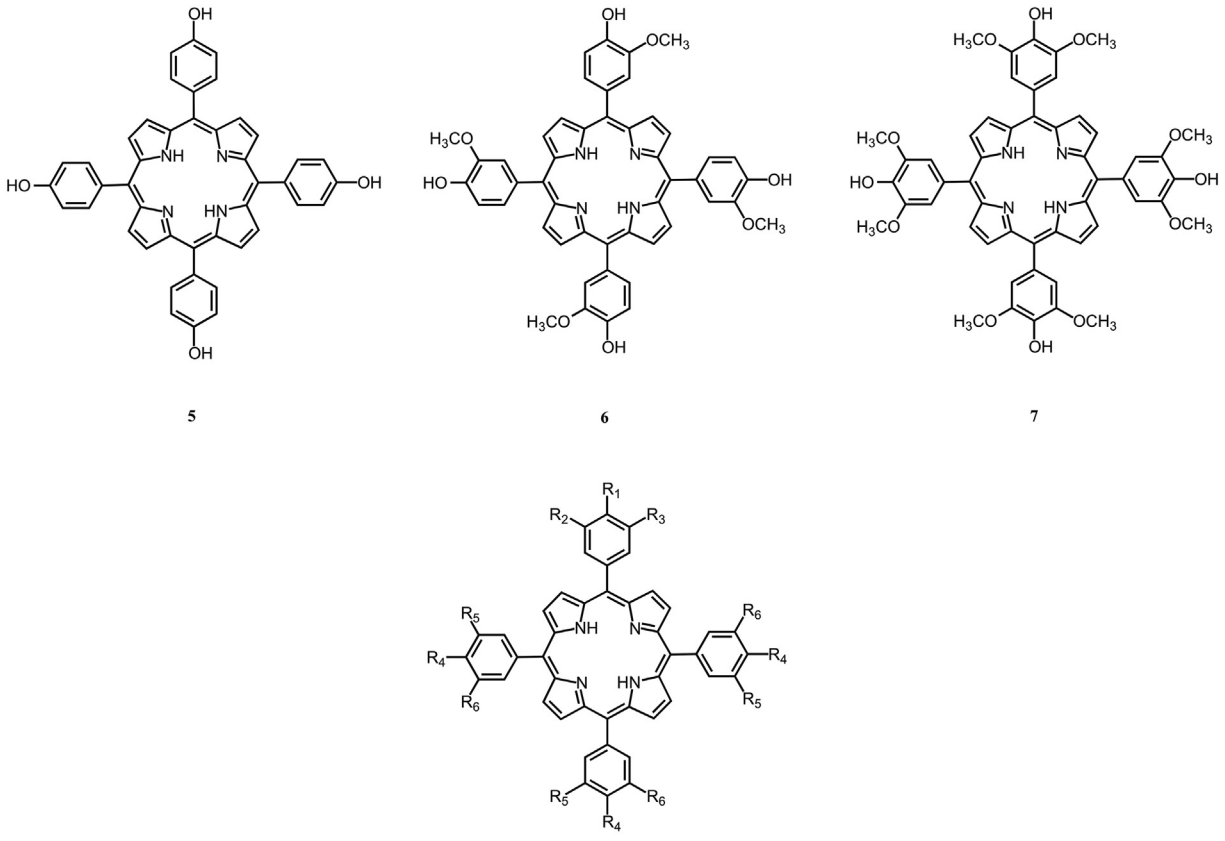


Fig. 3. Structures of investigated compounds.

An important parameter characterizing PDT usefulness of porphyrins is their ability to generate singlet oxygen. Determination of quantum yield of singlet oxygen production by all synthesized derivatives was carried out by laser flash photolysis. As a reference, we used tetrakisphenylporphyrin with a known quantum yield of singlet oxygen generation (0.62) [14]. Quantum yields of all the obtained compounds show very similar values.

2. Experimental

NMR spectra were obtained with a 400 MHz Bruker spectrometer in $CDCl_3$ or DMSO-d_6, and chemical shifts are reported in ppm downfield from TMS. ESI mass spectra were recorded on a Varian MS 500 or LCQ DUO FINNINGAN THERMOQUEST instruments using flow injection system. UV–Vis spectra were recorded in chloroform or methanol solutions using Genesys 6 (ThermoSpectronic) spectrophotometer. Fluorescence spectra were made with a Fluorescence Spectrophotometer Eclipse Cary Varian in the range 550–800 nm using 420 nm excitation wavelength and 1.0 nm the sampling interval for all compounds. Transient triplet–triplet absorption spectra were observed with an Applied Photophysics LKS 60

Table 1
Composition of compounds **8**–**19**.

Compound	R_1	R_2	R_3	R_4	R_5	R_6
8	$OC_{16}H_{33}$	H	H	OH	H	H
9	H	$OC_{16}H_{33}$	H	H	OH	H
10	$OCOC_{15}H_{31}$	H	H	OH	H	H
11	H	$OCOC_{15}H_{31}$	H	H	OH	H
12	$OC_{16}H_{33}$	OCH_3	H	OH	OCH_3	H
13	$OCOC_{15}H_{31}$	OCH_3	H	OH	OCH_3	H
14	$OC_{16}H_{33}$	OCH_3	OCH_3	OH	OCH_3	OCH_3
15	$OCOC_{15}H_{31}$	OCH_3	OCH_3	OH	OCH_3	OCH_3
16	$NHCOCH_3$	H	H	OCH_3	H	H
17	NH_2	H	H	OCH_3	H	H
18	$NHCOC_{15}H_{31}$	H	H	OCH_3	H	H
19	$NHCOC_{15}H_{31}$	H	H	OH	H	H

Table 2
Porphyrins **1**, **2**, **4**–**19** Soret band and Q bands wavelengths (λ nm) and log of molar extinction coefficients (log ε).

Porphyrin	Soret	$Q_y(1-0)$	$Q_y(0-0)$	$Q_x(1-0)$	$Q_x(0-0)$
	λ (log ε)	λ (log ε)	λ (log ε)	λ (log ε)	λ (log ε)
1	415 (5.66)	514 (4.25)	549 (3.81)	590 (3.62)	650 (3.67)
2	416 (5.28)	516 (4.03)	544 (3.87)	597 (3.65)	651 (4.41)
4	422 (5.54)	517 (4.16)	553 (3.81)	593 (3.66)	651 (3.70)
5	420 (5.63)	517 (4.21)	554 (3.90)	592 (3.65)	651 (3.72)
6	423 (5.59)	518 (4.18)	550 (3.80)	592 (3.65)	651 (3.65)
7	424 (5.61)	519 (4.19)	551 (3.77)	591 (3.71)	650 (3.55)
8	423 (5.52)	519 (4.11)	556 (3.91)	594 (3.63)	651 (3.67)
9	419 (5.60)	517 (4.15)	550 (3.79)	591 (3.62)	647 (3.66)
10	419 (5.55)	517 (4.16)	554 (3.88)	592 (3.68)	648 (3.71)
11	419 (5.51)	519 (4.17)	553 (3.90)	595 (3.61)	652 (3.69)
12	426 (5.56)	520 (4.18)	558 (3.94)	593 (3.69)	651 (3.68)
13	424 (5.53)	519 (4.19)	558 (3.89)	594 (3.59)	650 (3.61)
14	427 (5.54)	520 (4.20)	557 (3.87)	594 (3.70)	650 (3.58)
15	428 (5.39)	520 (4.14)	556 (3.84)	594 (3.72)	650 (3.61)
16	420 (5.55)	518 (4.13)	549 (3.84)	594 (3.69)	649 (3.53)
17	421 (5.56)	518 (4.15)	549 (3.83)	593 (3.67)	651 (3.54)
18	423 (5.51)	519 (4.09)	556 (3.81)	592 (3.69)	649 (3.62)
19	419 (5.58)	517 (4.21)	554 (3.82)	593 (3.63)	651 (3.66)

Table 3
Fluorescence spectra of compounds **1**, **2**, **4**–**19**: values for Q(0–0), Q(0–1), fluorescence quantum yields Φ_F (420 nm) and Stokes shifts between the Q(0–0) and Q_x(0–0) band.

Porphyrin	Q(0–0)	Q(0–1)	Φ_F	Stokes shift (nm)
	λ_{max} (nm)	λ_{max}(nm)		
1	654	719	0.098	4
2	651	715	0.210	0
4	651	717	0.096	0
5	656	721	0.123	5
6	656	724	0.126	5
7	657	725	0.120	7
8	657	720	0.126	6
9	648	713	0.086	1
10	653	718	0.114	5
11	650	713	0.092	−2
12	657	726	0.128	6
13	655	724	0.125	5
14	659	723	0.118	9
15	658	725	0.121	8
16	655	721	0.117	6
17	654	721	0.119	3
18	655	723	0.118	6
19	652	721	0.121	1

Laser Flash Photolysis Spectrometer, using the third harmonic (355 nm) of a Brilliant Nd-YAG Q-switched laser. The 150 W xenon lamp was used as the light source. Spectra were performed in the range from 350 to 800 nm with 1 nm interval. The quartz cell with optical length of 10 mm was used for all measurements. For flash photolysis and fluorescence measurements toluene or methanol solutions of compounds were used. All chemicals and solvents were purchased from Acros Organics.

*2.1. Tetrakis-5,10,15,20-(3-hydroxyphenyl)porphyrine (**1**) ($C_{44}H_{30}N_4O_4$ = 678.734)*

A solution of 3-hydroxybenzaldehyde (8.8 g, 72 mmol) in propionic acid (300 mL) was brought to reflux. Pyrrole (5 mL, 72 mmol) was added quickly. The resulting mixture was refluxing during additional 1.5 h. After that 200 mL of propionic acid was evaporated. Cooled to room temperature residue was neutralized with saturated solution of $NaHCO_3$. Precipitated crude porphyrin was washed with chloroform and finally chromatographed on silica gel column with dichloromethane-methanol (30:1, v/v) mixture as eluent to give 1.11 g (9%) of **1**.

^{1}H NMR (400 MHz, DMSO-d_6): δ(ppm): 10.00 (s, 4H, O*H*), 8.88 (s, 8H, β*H*), 7.66–7.56 (m, 12H, Ar*H*), 7.27–7.21 (m, 4H, Ar*H*), −3.02 (s, 2H, N*H*); ESI MS (m/z): 680.1 $[M + H]^+$.

Tetrakis-5,10,15,20-(3-hydroxyphenyl)chlorine (**2**) was obtained according to [15].

Table 4
Triplet state lifetimes τ_T values (μs) in the presence and absence of oxygen and quantum yield of singlet oxygen Φ_Δ for compounds **1**, **2** and **4**–**19**.

Porphyrin	τ_T [μs] [oxygen]	τ_T [μs] [oxygen-free]	Φ_Δ
1	0.275	2.00	0.60
2	0.219	1.27	0.57
4	0.257	1.48	0.60
5	0.231	2.00	0.61
6	0.258	1.90	0.62
7	0.324	2.01	0.63
8	0.232	1.77	0.64
9	0.279	1.35	0.63
10	0.237	1.13	0.65
11	0.266	1.80	0.64
12	0.265	1.43	0.68
13	0.271	1.33	0.62
14	0.276	1.48	0.69
15	0.300	1.20	0.62
16	0.273	1.81	–
17	0.252	1.70	–
18	0.286	1.20	0.61
19	0.217	1.27	0.67

*2.2. 5-(4-Hexadecyloxyphenyl)-10,15,20-tris(3-hydroxyphenyl) porphyrine (**4**) ($C_{60}H_{62}N_4O_4$ = 903.159)*

3-Hydroxybenzaldehyde (3.66 g, 0.03 mol), 4-hexadecyloxybenzaldehyde (3.46 g, 0.01 mol) were dissolved in 100 mL of propionic acid and heated to reflux. Pyrrole (2.8 mL, 0.04 mol) was added and the resulting mixture was heated under reflux for 1.5 h. 50 mL of propionic acid was removed by distillation. 200 mL of water was added to the rest of cooled mixture. Propionic acid was neutralized by solid sodium carbonate. Precipitated organic compounds were dissolved in dichloromethane (500 mL), washed with water (5 × 100 mL) and dried with anhydrous Na_2SO_4. Dichloromethane was evaporated and the residue was chromatographed on silica gel with chloroform–ethyl acetate (3:1, v/v) mixture as eluent. Yield: 0.34 g (~4%).

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.99 (s, 3H, O*H*), 8.87 (m, 8H, β*H*), 8.05 (d, 2H, *J* = 7.2 Hz, Ar*H*), 7.67–7.55 (m, 8H, Ar*H*), 7.33, 7.20 (dd, 6H, Ar*H*), 4.15 (t, 2H, O$CH_2$$CH_2$), 1.81 (q, 2H, O$CH_2$$CH_2$), 1.47 (q, 2H), 1.40–1.05 (m, 24H), 0.76 (t, 3H, $CH_2$$CH_3$), −2.99 (s, 2H, N*H*); ESI MS (m/z): 904.8 $[M + H]^+$.

*2.3. Tetrakis-5,10,15,20-(4-hydroxyphenyl)porphyrine (**5**) ($C_{44}H_{30}N_4O_4$ = 678.734)*

This compound was obtained similar to **1**, using 4-hydroxybenzaldehyde instead of 3-hydroxybenzaldehyde. Yield: 12%.

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 8.93 (s, 8H, β*H*), 8.87 (s, 4H, O*H*), 8.07 (d, 8H, *J* = 8.0 Hz, Ar*H*), 7.30 (d, 8H, *J* = 8.0 Hz, Ar*H*), −2.68 (s, 2H, N*H*); ESI MS (m/z): 679.3 $[M + H]^+$.

*2.4. Tetrakis-5,10,15,20-(4-hydroxy-3-methoxyphenyl)porphyrine (**6**) ($C_{48}H_{38}N_4O_8$ = 798.27)*

This compound was obtained similar to **1**, using 4-hydroxy-3-methoxybenzaldehyde (vanillin) instead of 3-hydroxybenzaldehyde. Product was chromatographed on silica gel with chloroform:ethyl acetate (5:1, v/v). Yield: 22%.

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.53 (s, 4H, O*H*), 8.91 (s, 8H, β*H*), 7.77 (s, 4H, Ar*H*), 7.58 (d, 4H, *J* = 8.0 Hz, Ar*H*), 7.21 (d, 4H, *J* = 8.0 Hz, Ar*H*), 3.86 (s, 12H, OCH_3), −2.88 (s, 2H, N*H*); ESI MS (m/z): 798.6 $[M + H]^+$.

*2.5. Tetrakis-(4-hydroxy-3,5-dimethoxyphenylene)porphyrine (**7**) ($C_{52}H_{46}N_4O_{12}$ = 918.94)*

This compound was obtained similar to **6**, using 4-hydroxy-3,5-dimethoxybenzaldehyde instead of 4-hydroxy-3-methoxybenzaldehyde. Yield: 22%.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.95 (s, 8H, β*H*), 7.48 (s, 8H, Ar*H*), 5.90 (s, 4H, O*H*), 4.01 (s, 24H, OCH_3), −2.74 (s, 2H, N*H*); ESI MS (m/z): 919.7 $[M + H]^+$.

*2.6. 5-(4-Hexadecyloxyphenyl)-10,15,20-tris(4-hydroxyphenyl) porphyrine (**8**) ($C_{60}H_{62}N_4O_4$ = 903.16)*

A 0.262 g (0.386 mmol) of **5** was stirred in 5 mL dimethylformamide with 0.024 g (0.579 mmol) 60% NaH for 30 min at

room temperature. 0.118 g (0.386 mmol) of 1-bromohexadecane was added. The reaction mixture was stirred for 24 h at 80–90 °C. The solvent was evaporated in vacuum then water (100 mL) was added and the resulting mixture was extracted three times (3 × 50 mL) with dichloromethane-ethyl acetate (3:2) mixture. Combined organic fraction was washed with water (3 × 100 mL), dried with anhydrous Na_2SO_4. Solvents were evaporated and the residue was chromatographed on silica gel with chloroform-ethyl acetate (5:1, v/v). Yield: 0.056 g (16%).

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.99 (s, 3H, O*H*), 8.88 (m, 8H, β*H*), 7.99 (d, 8H, *J* = 8.0 Hz, Ar*H*), 7.21 (d, 8H, *J* = 8.0 Hz, Ar*H*), 4.11 (t, 2H, OC*H*$_2$CH$_2$), 1.83–1.71 (m, 2H, OCH$_2$C*H*$_2$), 1.50–1.39 (m, 2H, OCH$_2$CH$_2$C*H*$_2$), 1.29–1.10 (m, 24H, C*H*$_2$), 0.78 (t, 3H, C*H*$_3$), −2.85 (s, 2H, N*H*); ESI MS (m/z): 905.0 $[M + H]^+$.

2.7. 5-(3-Hexadecyloxyphenyl)-10,15,20-tris(3-hydroxyphenyl) porphyrine (**9**) ($C_{60}H_{62}N_4O_4$ = 903.16)

This compound was obtained similar to **8**, using porphyrine **1** as a parent substance. Yield: 14%.

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.87 (s, 3H, O*H*), 8.88 (m, 8H, β*H*), 7.76 (m, 2H, Ar*H*), 7.69 (t, 1H, Ar*H*), 7.65–7.55(m, 9H, Ar*H*), 7.39 (d, 1H, Ar*H*), 7.24 (d, 3H, Ar*H*), 4.16 (t, 2H, OC*H*$_2$CH$_2$), 1.79 (q, 2H, OCH$_2$C*H*$_2$), 1.44 (q, 2H), 1.35–1.00 (m, 24H), 0.76 (t, 3H, CH$_2$C*H*$_3$), −2.97 (s, 2H, N*H*); ESI MS (m/z): 905 $[M + H]^+$.

2.8. [5-(4-Oxyphenyl)-10,15,20-tris(4-hydroxyphenyl)porphyrine] palmitate (**10**) ($C_{60}H_{60}N_4O_5$ = 917.14)

0.212 g (0.313 mmol) of **5** was stirred in 5 mL dimethylformamide with 0.082 g (0.3 mmol) of palmitoyl chloride. The reaction mixture was stirred for 48 h at 80 °C. Water (100 mL) and triethylamine (2 mL) were added and the resulting mixture was extracted four times (4 × 50 mL) with chloroform-ethyl acetate (3:2) mixture. Combined organic fraction was washed with water (5 × 50 mL), dried with anhydrous Na_2SO_4. Solvents were evaporated and the residue was chromatographed on silica gel with chloroform-ethyl acetate (5:1, v/v). Yield: 0.061 g (22%).

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.95 (s, 3H, O*H*), 8.88 (m, 6H, β*H*), 8.70 (d, 2H, β*H*), 8.23 (d, 2H, *J* = 8.8 Hz, Ar*H*), 8.00 (d, 6H, *J* = 8.4 Hz, Ar*H*), 7.56 (d, 2H, *J* = 8.8 Hz, Ar*H*), 7.20 (d, 6H, *J* = 8.4 Hz, Ar*H*), 2.75 (t, 2H), 1.79 (q, 2H), 1.71 (q, 2H), 1.60 (q, 2H), 1.30–1.18 (m, 20H), 0.80 (t, 3H, C*H*$_3$), −2.88 (s, 2H, N*H*). ESI MS (m/z): 919.0 $[M + H]^+$.

2.9. [5-(3-Oxyphenyl)-10,15,20-tris(3-hydroxyphenyl)porphyrine] palmitate (**11**) ($C_{60}H_{60}N_4O_5$ = 917.14)

This compound was obtained similar to **10**, using porphyrine **1** as a parent substance. Yield: 18%.

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 9.91 (s, 3H, O*H*), 8.89 (s, 8H, β*H*), 8.12 (d, 1H, Ar*H*), 7.98 (t, 1H, Ar*H*), 7.85 (t, 1H, Ar*H*), 7.75–7.55 (m, 9H, Ar), 7.24 (d, 4H, Ar*H*), 1.69 (t, 2H), 1.34–0.97 (m, 26H), 0.77 (t, 3H), −2.98 (s, 2H, N*H*). ESI MS (m/z): 918.9 (100) $[M + H]^+$.

2.10. 5-(4-Hexadecyloxy-3-methoxyphenyl)-10,15,20-tris(4-hydroxy-3-methoxyphenyl)porphyrine (**12**) ($C_{64}H_{70}N_4O_8$ = 1023.26)

This compound was obtained similar to **8**, using porphyrine **6** as a parent substance. Yield: 11%.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.97 (s, 8H, β*H*), 7.82 (s, 1H, Ar*H*), 7.80–7.74 (m, 8H, ArH), 7.34 (d, 3H, Ar*H*), 6.02 (s, 3H, O*H*), 4.34 (t, 2H), 4.04 (s, 9H, OC*H*$_3$), 4.01 (s, 3H, OC*H*$_3$), 2.12–2.04 (m, 2H), 1.70–1.62 (m, 2H), 1.40–1.24 (m, 24H), 0.92 (t, 3H), −2.70 (s, 2H, N*H*). ESI MS (m/z): 1024.2 $[M + H]^+$.

2.11. [5-(3-Methoxy-4-oxyphenyl)-10,15,20-tris(4-hydroxy-3-methoxyphenyl)porphyrine] palmitate (**13**) ($C_{64}H_{68}N_4O_9$ = 1036.50)

This compound was obtained similar to **10**, using porphyrine **6** as a parent substance. Yield: 12%.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.97 (s, 8H, β*H*), 7.89–7.81 (m, 9H, ArH), 7.34 (d, 3H, Ar*H*), 6.00 (s, 3H, O*H*), 4.06 (s, 9H, OC*H*$_3$), 4.01 (s, 3H, OC*H*$_3$), 1.69 (t, 2H), 1.30–0.97 (m, 26H), 0.77 (t, 3H), −2.71 (s, 2H, N*H*). ESI MS (m/z): 1037.2 $[M + H]^+$.

2.12. 5-(4-Hexadecyloxy-3,5-dimethoxyphenyl)-10,15,20-tris(4-hydroxy-3,5-dimethoxyphenyl) porphyrine (**14**) ($C_{68}H_{78}N_4O_{12}$ = 1143.37)

This compound was obtained similar to **8**, using porphyrine **7** as a parent substance. Yield: 22%.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 9.00 (s, 8H, β*H*), 7.54 (s, 6H, Ar*H*), 7.52 (s, 2H, Ar*H*), 5.98 (s, 3H, O*H*), 4.37 (t, 2H), 4.07 (s, 18H, OC*H*$_3$), 4.01, (s, 6H, OC*H*$_3$), 2.08–1.98 (m, 2H), 1.70 (m, 2H), 1.40–1.28 (m, 24H), 0.92 (t, 3H), −2.69 (s, 2H, N*H*). ESI MS (m/z): 1145.0 $[M + H]^+$.

2.13. [5-(3,5-Dimethoxy-4-oxyphenyl)-10,15,20-tris(4-hydroxy-3,5-dimethoxyphenyl)porphyrine] palmitate (**15**) ($C_{68}H_{76}N_4O_{13}$ = 1156.54)

This compound was obtained similar to **10**, using porphyrine **7** as a parent substance. Yield: 19%.

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.99 (s, 8H, β*H*), 7.56 (s, 6H, Ar*H*), 7.50 (s, 2H, Ar*H*), 5.95 (s, 3H, O*H*), 4.11 (s, 18H, OC*H*$_3$), 4.03 (s, 6H, OC*H*$_3$), 1.63 (t, 2H), 1.33–1.01 (m, 26H), 0.79 (t, 3H), −2.68 (s, 2H, N*H*). ESI MS (m/z): 1157.6 $[M + H]^+$.

2.14. 5-(4-Acetylaminophenyl)-10,15,20-tris(4-methoxyphenyl) porphyrine (**16**) ($C_{49}H_{39}N_5O_4$ = 761.86)

4-Acetamidbenzaldehyde (3.26 g, 0.02 mol), 4-methoxybenzaldehyde (8.16 g, 0.06 mol) were dissolved in 100 mL of propionic acid and heated to reflux. Pyrrole (5.6 mL, 0.08 mol) was added and the resulting mixture was heated under reflux for 1.5 h. 50 mL of propionic acid was removed by distillation. 200 mL of water was added to the rest of cooled mixture. Propionic acid was neutralized by solid sodium carbonate. Precipitated organic compounds were dissolved in dichloromethane (400 mL), washed with water (3 × 150 mL) and dried with anhydrous Na_2SO_4. Dichloromethane was evaporated and the residue was chromatographed on silica gel with chloroform–ethyl acetate (3:1, v/v) mixture as eluent. Yield: 0.457 g (~3%).

^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.87 (m, 8H, β*H*), 8.47 (d, 2H, *J* = 8.0 Hz, Ar*H*), 8.13 (d, 6H, *J* = 8.8 Hz, Ar*H*), 7.86 (d, 2H, *J* = 8.0 Hz, Ar*H*), 7.29 (d, 6H, *J* = 8.8 Hz, Ar*H*), 4.10 (s, 9H, OC*H*$_3$), 2.10 (s, 3H, C*H*$_3$), −2.75 (s, 2H, N*H*).

ESI MS (m/z): 762.7 $[M + H]^+$.

2.15. 5-(4-Aminophenyl)-10,15,20-tris(4-methoxyphenyl)porphyrine (**17**) ($C_{47}H_{37}N_5O_3$ = 719.83)

0.21 g (0.276 mmol) of **16** was dissolved in the mixture of 20 mL of trifluoroacetic acid and 21 mL of concentrated hydrochloric acid and heated at 80 °C with stirring during 20 h. 200 mL of water was added to the rest of cooled mixture. Precipitated green organic compounds were dissolved in chloroform-ethyl acetate mixture (300 mL; 3:1, v/v), washed with water (100 mL), saturated sodium carbonate solution (2 × 100 mL), water (100 mL) and dried with anhydrous Na_2SO_4. Solvent was evaporated. Yield: 0.145 g (73%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.92 (d, 2H, β*H*), 8.86 (m, 6H, β*H*), 8.12 (d, 6H, *J* = 8.4 Hz, Ar*H*), 7.99 (d, 2H, *J* = 8.0 Hz, Ar*H*), 7.29 (d, 6H,

J = 8.4 Hz, Ar*H*), 7.06 (d, 2H, J = 8.0 Hz, Ar*H*), 4.10 (s, 9H, O*CH*$_3$), −2.73 (s, 2H, N*H*); ESI MS (m/z): 721.2 (100) $[M + H]^+$.

2.16. N-[5-(para-phenylene)-10,15,20-tris(4-methoxyphenyl) porphyrine] palmitylamide (**18**) ($C_{63}H_{67}N_5O_4$ = 958.23)

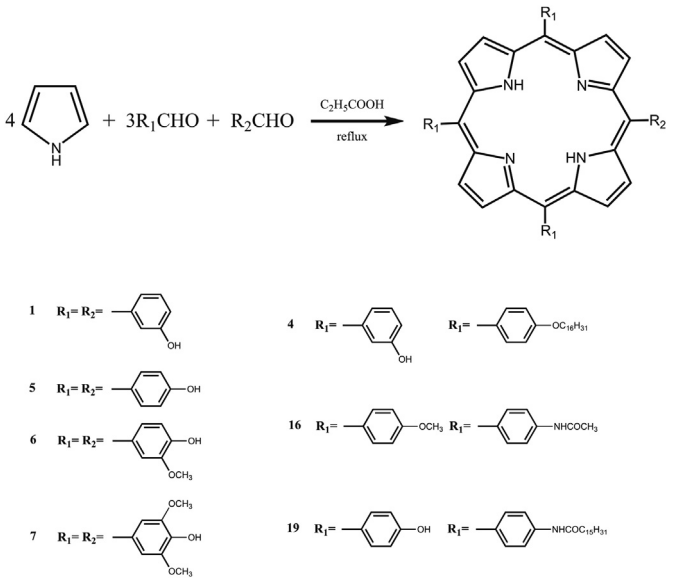
Palmitic acid 0.128 g (0.5 mmol) and 0.5 mL of 1 M DCC solution (0.5 mmol) dissolved in 10 mL dichloromethane were refrigerated for 4 days. After that 0.030 g (0.042 mmol) of **17** dissolved in 10 mL of dichloromethane was added and the resulting mixture was stirred for 24 h at room temperature. 50 mL of dichloromethane was added to the mixture and resulting solution was washed with water (3 × 50 mL) and dried with anhydrous Na_2SO_4. Dichloromethane was evaporated and the residue was chromatographed on silica gel with dichloromethane–methanol (100:1, v/v) mixture as eluent. Yield: 0.0293 g (73%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.87 (m, 8H, β*H*), 8.13 (d, 2H, J = 8.4 Hz, Ar*H*), 8.12 (d, 6H, J = 8.0 Hz, Ar*H*), 7.88 (d, 2H, J = 8.4 Hz, Ar*H*), 7.28 (d, 6H, J = 8.0 Hz, Ar*H*), 4.09 (s, 9H, O*CH*$_3$), 2.50 (t, 2H), 1.86 (q, 2H), 1.47 (q, 2H), 1.40–1.20 (m, 22H), 0.89 (t, 3H), −2.74 (s, 2H); ESI MS (m/z): 959.8 $[M + H]^+$.

2.17. N-[5-(para-phenylene)-10,15,20-tris(4-hydroxyphenyl) porphyrine] palmitylamide (**19**) ($C_{60}H_{61}N_5O_4$ = 915.47)

N-(4-formylphenyl)amide of palmitic acid (2.39 g, 6.66 mmol), 4-hydroxybenzaldehyde (2.44 g, 20 mmol) were dissolved in 100 mL of propionic acid and heated to reflux. Pyrrole (1.9 mL, 27 mmol) was added and the resulting mixture were heated under reflux for 1.5 h. 50 mL of propionic acid was removed by distillation. 200 mL of water was added to the rest of cooled mixture. Propionic acid was neutralized by solid sodium carbonate. Precipitated porphyrin mixture was separated and dried on air. Pure **19** was separated by column chromatography on silica gel with chloroform–ethyl acetate (5:1, v/v) mixture as eluent. Yield: 0.152 g (~2.5%).

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) 10.31 (s, 1H, N*H*), 9.97 (s, 3H, O*H*), 8.89 (s, 8H, β*H*), 8.14 (d, 2H, J = 8.0 Hz, Ar*H*), 8.08 (d, 2H, J = 8.0 Hz, Ar*H*), 8.02 (d, 6H, J = 8.0 Hz, Ar*H*), 7.23 (d, 6H, J = 8.0 Hz, Ar*H*), 1.71 (t, 2H), 1.28-1.12 (m, 26H), 0.79 (t, 3H), -2.85 (s, 2H, N*H*). ESI MS (m/z): 916.2 $[M + H]^+$ (Scheme 1).



Scheme 1. Porphyrin synthesis via Adler-Longo method.

3. Results and discussion

3.1. Porphyrin synthesis

Compounds **1**, **4**–**7**, **16** and **19** were synthesized by condensation of appropriate aldehydes with pyrrole in propionic acid in typical Adler-Longo procedure. Compound **17** was obtained from **16** by acid (trifluoroacetic acid) hydrolysis. Compound **2** was obtained from **1** using procedure described in [15].

Ether derivatives of porphyrins **8**, **9**, **12** and **14** were obtained from porphyrins **5**, **1**, **6** and **7**, respectively, in a typical Williamson ether synthesis. The reaction was carried out in DMF using NaH to obtain suitable phenoxide salts of porphyrins, and cetyl bromide as an alkylating agent. Compared with post-cyclisation reaction, more convenient method for synthesis of compound **4** and **19** is mixed Adler-Longo condensation (Scheme 1). It eliminates possibility of non separable isomers formation. E.g. Derivatisation of 5-(4-hydroxyphenyl)-10,15,20-tris(3-hydroxyphenyl)-porphyrin obtained first via 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde and pyrrole condensation would lead to 5-(4-hexadecyloxyphenyl)-10,15,20-tris(3-hydroxyphenyl)-porphyrin and 5-(3-hexadecyloxyphenyl)-10,15,20-tris(3-hydroxyphenyl)porphyrin and mixture of such compounds seems to impossible to separate. Thus, mixed condensation of 4-hexadecyloxybenzaldehyde, 3-hydroxybenzaldehyde and pyrrole was performed to obtain compound 4. Other compounds e.g. **8** or **9** could be either obtains by this method or by post-cyclization derivatisation. Palmitic acid esters **10**, **11**, **13** and **15** were obtained from porphyrins **5**, **1, 6** and **7**, respectively, by acylation with palmitic acid chloride (Scheme 2). In our experiments we tried to obtain these esters from suitable porphyrin and acid using DCC and DMAP, but it turned out that using acid chlorides leads to better yields of final compounds and in shorter time periods. Amide **18** was obtained from **17** and palmitic acid in the presence of DCC and DMAP in dichloromethane. Generally, one can conclude that introduction of long alkyl chain into porphyrin molecule considerably ameliorates its solubility in organic solvents which is useful during later product purification and spectroscopic studies of these compounds.

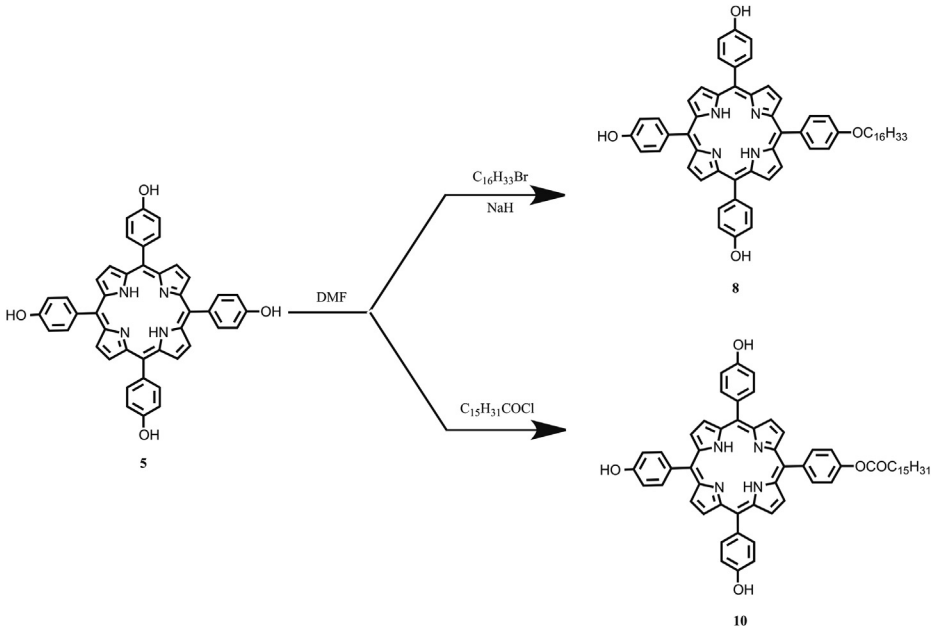
3.2. Mass spectra

ESI MS mass spectra performed by direct sample introduction into ionizing chamber demonstrated high purity of the synthesized compounds. In all cases single $[M + H]^+$ peaks (of the highest intensity) were present. Besides these peaks, $[2M + H]^+$ ion peaks were sometimes observed, corresponding to porphyrin aggregates, as well as $[M + Na]^+$ and $[2M + Na]^+$ ion peaks, but of considerably lesser intensity. Addition of sodium salts (any) to porphyrin solutions immediately increased intensity of $[M + Na]^+$ ion which became one with the highest intensity. Similar effect was observed with other alkaline ions (following addition of their salts).

3.3. Lipophilicity

Lipophilicity of the majority of compounds described herein was the subject matter of our previous report [16]. It is an important parameter from the perspective of applying these compounds to prepare liposomes (i.e. to incorporate the long alkyl chain into the liposomal bilayer).

According to expectations, attachment of alkyl chain to porphyrin molecule considerably increased its hydrophobic properties and positively affected its solubility in various organic solvents. The manner in which long alkyl chain was attached to phenylporphyrin is not insignificant. Ether bond most strongly increased hydrophobic properties of the obtained compounds; however, from the biological perspective, such bond between the



Scheme 2. Reaction of attaching the alkyl chain to the porphyrin via ether and ester bond.

two moieties is not the best solution, especially in the context of later catabolism of the molecule. Ester or amide bonds are much better in this respect but they tend to increase the hydrophilic character of the molecule (compared to one with ether bond). The consideration that the ester or amide bond is better then the ether bond, based on the metabolic stability of the compound. It is general rule that compounds with reducted lipophilicity are more stable [17]. We already proved that compounds with alkyl chain attached via ether bond are more lipophylic then these where the alkyl chain is attached via ester or amide bond. Therefore esters or amides could be easier removed from the organism after photodynamic action. We do not have data at this point concerning "biological response" and usefulness of compounds differing by bond type between hydrophilic and hydrophobic fragments of porphyrin derivatives. Such knowledge requires separate biological studies of these compounds.

3.4. Absorption spectroscopy

Absorption spectra were measured between 300 and 800 nm in chloroform solutions using a 1 cm cuvette and a 1 nm slit width. Solutions were made at concentrations 10^{-6} M and 10^{-4} M for Soret band and Q bands regions, respectively.

Table 2 shows data concerning spectral properties of all the synthesized porphyrin derivatives. They are very similar to those of known tetrakisphenylporphyrin with a Soret band at 420 nm, and four less intense Q bands around 520, 550, 590 and 650 nm [18]. Particular compounds show minute differences between maxima and intensities of absorption bands.

Comparison of the porphyrins' spectra leads to the conclusion that they are not affected by the type and nature of the long alkyl chain in the molecule.

Table 2 also shows spectrum of chlorin **2** which basically does not differ from the remaining studied compounds. The greatest change in the spectrum concerns intensity of the last $Q_x(0-0)$ band.

3.5. Fluorescence spectra

Fluorescence experiments were performed at room temperature using Eclipse Cary (Varian) spectrofluorimeter. For fluorescence emission the sample, deaerated with argon for 30 min, was excited at 420 nm. Porphyrin fluorescence quantum yields (Φ_F) were determined in dilute solutions (absorption range 0.02–0.05) in the Soret band region using tetrakisphenylporphyrin ($\Phi_F = 0.11$) as a standard [19].

The fluorescence quantum yield was found using equation:

$$\varphi = \varphi_w \frac{\int_0^\infty I_f(\lambda)d\lambda}{\int_0^\infty I_f^w(\lambda)d\lambda} \cdot \frac{1-10^{-A_w}}{1-10^{-A}} \cdot \frac{n^2}{n_w^2}$$

where $\int_0^\infty I_f(\lambda)d\lambda$ and $\int_0^\infty I_f^w(\lambda)d\lambda$ are areas under the emission curves, A and A_w are absorbances of the sample and standard, n and n_w are refractive indexes of solvents used to dissolution the sample and standard respectively.

Fluorescence spectra of all the obtained porphyrins show similar shapes with maxima at 650 and 720 nm (Fig. 4). Fluorescence quantum yields (Φ_F) of all the porphyrins studied are relatively small; this shows that the studied compounds might be promising PDT agents. The yields for all the compounds studied are lower than that of chlorin **2** which has been used as photosensitizer. Stokes shift which characterizes the magnitude of shift between Q(0–0) band of the fluorescence spectrum and $Q_x(0-0)$ band of the absorption spectrum are minute in the case of the compounds studied and this likely reflects minor structural alterations occurring in porphyrin molecules upon excitation [20].

3.6. Triplet state lifetimes and singlet oxygen quantum yields

Table 4 shows data concerning the most important photochemical parameters from the standpoint of PDT usefulness. They are triplet state lifetimes (τ_T) of the photosensitizer in the absence and presence of oxygen and singlet oxygen quantum yields (Φ_Δ). The triplet lifetimes for the investigated compounds were determined using laser flash photolysis spectrometry. Absorbance of the solutions was in the 0.2–0.3 range at 355 nm. The maximum of

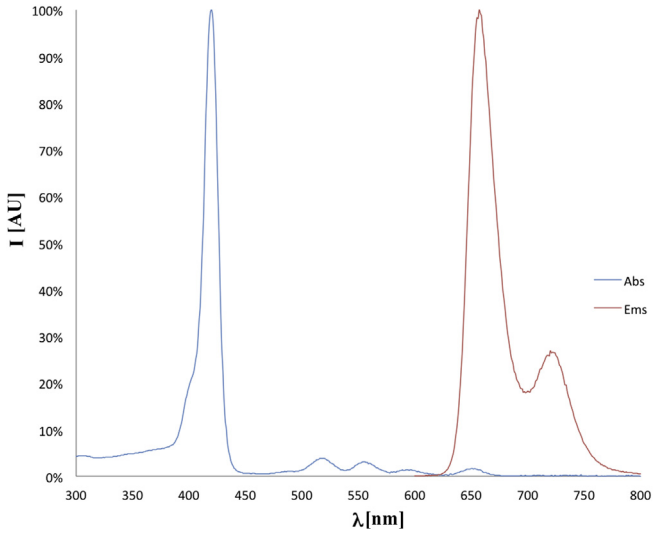


Fig. 4. Absorption and emission spectra of compound **8.**

triplet–triplet absorption was determined from transient absorption measurement (for the investigated compounds the triplet–triplet transition maximum was in the 440–470 nm range). The triplet lifetimes were determined for aerated and deaerated samples. After sample excitation the decay curves were observed (Fig. 5). All decays were fitted to monoexponential which is in agreement with literature data [21].

In order for the compound to be a good photosensitizer it should have high quantum yield of triplet state lifetime. A compound that remains in the triplet state for a relatively long time can transfer its energy to other molecules present in the vicinity; in case of PDT these are oxygen molecules which, as a result of this process become the actual therapeutic agent.

Table 4 lists triplet state lifetimes for particular compounds; the lifetimes are averaged from ten measurements. Extended triplet state lifetimes are noted for lack of oxygen presence conditions which suggests effective quenching of triplet states by oxygen when present in the sample at the time of measurement. Also in this case it can be seen that triplet state lifetimes for the majority of obtained compounds are distinctively longer than that for Foscan (**2**).

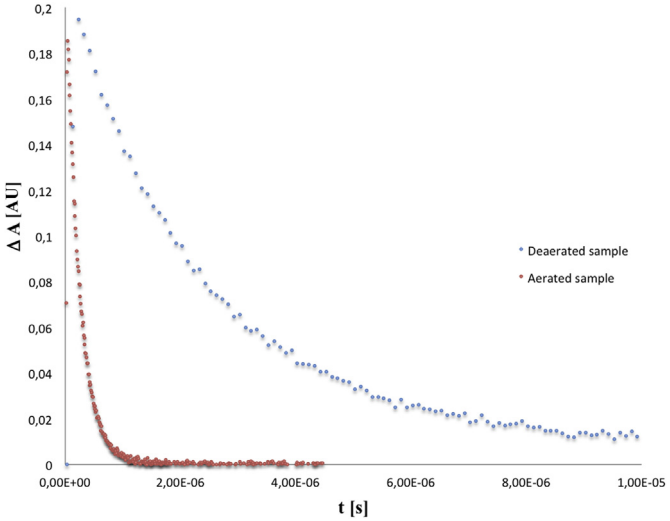


Fig. 5. Triplet state decay curves: aerated and deaerated.

There are numerous methods available to determine quantum field of singlet oxygen; these were discussed in [14].

In our experiments the singlet oxygen quantum yields were determined using laser flash photolysis spectroscopy. The procedure employs values of oxygen luminescence measured by different laser energies for sample and standard (Fig. 6). The sample and standard absorbance were adjusted to 0.2. The characteristic singlet oxygen phosphorescence was observed at 1270 nm after laser pulse. The oxygen luminescence intensities were extrapolated to time-zero and the singlet oxygen quantum yields were determined from the ration of the slopes obtained for investigated compound and standard. Whole procedure is described in literature [22]. Phenalenone and tetrakisphenylporphyrin were used as reference and their singlet oxygen quantum yields are 0.95 and 0.7, respectively [14].

As can be seen from Table 4 quantum yields of singlet oxygen are comparable for all the porphyrins studied as well as with such yield for Foscan. As can be noticed, presence of additional substituents in phenyl fragments does not influence the process of energy transfer from photosensitizer to oxygen molecules. Quantum yield of singlet oxygen (0.59) reported in the literature for Foscan [23] is comparable to that determined in our study (0.57) and to that of Photofrin 0.2 [24]. It can be thus deduced that the studied porphyrins meet necessary requirements for potential photosensitizers useful for PDT.

3.7. *Photostability*

This is an additional important parameter of any potential photosensitizer. It should be stable in the dark. Upon light exposure it should go into an excited state, transfer its energy to neighboring molecules (for example oxygen) and return to ground state. It can so happen that as a result of such processes photosensitizer molecule will be degraded and shall lose its photosensitizing properties. Such process can be influenced by numerous external factors such as the solvent, substituents present or the compound's aggregation. In our study we determined stability of synthesized compound by using UV–Vis spectroscopy. We measured Soret band and Q bands absorbance values prior to and after light irradiation. Dose and dose rate of irradiation were chosen in such a way as to correspond to values that may occur in a biological setting. It turns out that all of the synthesized compounds are stable under such conditions. Only in the case of compound **2** loss of absorbance was observed after irradiation but after 60 min the absorbance was still at 90% level of the initial value. Photosensitizer solutions were

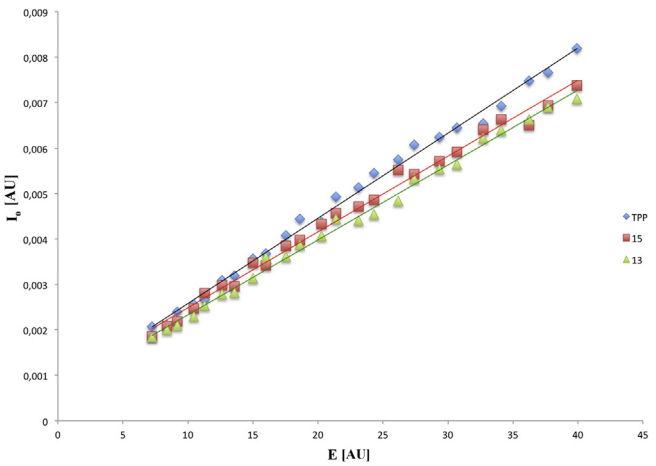


Fig. 6. Dependence of oxygen luminescence and laser energies.

kept in the dark (4 °C) for 30 days and no decay in absorbance values was found after that time. This suggests thus under these conditions the examined compounds were stable and probably did not undergo aggregation.

4. Conclusion

A series of porphyrin derivatives was synthesized for potential use in PDT or PDD. Their photochemical properties are akin to those of tetrakis(3-hydroxyphenyl)-porphyrin, the source compound of Foscan, widely used in PDT. Our further studies upon these compounds will likely involve synthesis of chlorin and bacteriochlorin derivatives. So far we were able to obtain two such derivatives but as an isomer mixtures. In the case of single substitution of the starting symmetrical porphyrins one obtains two different compounds which are very often difficult to separate. Further studies involving choice of suitable liposomal formulations for biological transfer of the obtained photosensitizers are underway.

Acknowledgments

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References

[1] Zięba G, Rojkiewicz M, Kozik V, Jarzembek K, Jarczyk A, Sochanik A, et al. The synthesis of new potential photosensitizers. 1. Mono-carboxylic acid derivatives of tetraphenylporphyrin. Monatshefte für Chemie 2012;143:153–9.
[2] Yano S, Hirohara S, Obata M, Hagiya Y, Ogura S, Ikeda A, et al. Current states and future views in photodynamic therapy. Journal of Photochemistry and Photobiology C: Photochemistry Reviews 2011;12:46–67.
[3] Bonnett R, McGarvey DJ, Harriman A, Land EJ, Truscott TG, Winsfield U-J. Photophysical properties of meso-Tetraphenylporphyrin and some meso-Tetra(hydroxyphenyl)porphyrins. Photochemistry and Photobiology 1988;48:271–6.
[4] Berenbaum MC, Akande SL, Bonnett R, Kaur H, Ionnou S, White RD, et al. meso-Tetra(hydroxyphenyl)porphyrins, a new class of potent tumour photosensitisers with favourable selectivity. British Journal of Cancer 1986;54:717–25.
[5] Bonnett R. Photodynamic therapy. Reviews Contemporary Pharmacotherapy 1999;10:1–17.
[6] Ma L, Moan J, Berg K. Evaluation of a New photosensitizer, meso-tetrahydroxyphenylchlorin, for use in photodynamic therapy: a comparison of its photobiological properties with those of two other photosensitizers. International Journal of Cancer 1994;57:883–8.
[7] Bonnett R, Charlesworth P, Djelal BD, Foley S, McGarvey DJ, Truscott TG. Photophysical properties of 5,10,15,20-tetrakis(m-hydroxyphenyl)porphyrin (m-THPP), 5,10,15,20-tetrakis(m-hydroxyphenyl)chlorine (m-THPC) and 5,10,15,20-tetrakis(m-hydroxyphenyl)-bacteriochlorin (m-THPBC): a comparative study. Journal of the Chemical Society, Perkin Transactions 2 1999:325–8.
[8] Berenbaum MC, Bonnett R, Chevretton EB, Akande-Adebakin SL, Ruston M. Selectivity of meso-Tetra(hydroxyphenyl)porphyrins and chlorins and of Photofrin II in causing photodamage in tumour, skin, muscle and bladder. The concept of cost-benefit in analysing the results. Lasers in Medical Science 1993;8:235–43.
[9] Dong P, Choi P, Schmiedl UP, Nelson JA, Starr FL, Ho RJY. Interaction of manganese–mesoporphyrin with oleic acid vesicles. Biochemistry 1995;34:3416–22.
[10] Guo L. Side-chain-controlled H- and J-aggregation of amphiphilic porphyrins in CTAB micelles. Journal of Colloid and Interface Science 2008;322:281–6.
[11] Bronshtein I, Smith KM, Ehrenberg B. The effect of pH on the topography of porphyrins in lipid membranes. Photochemistry and Photobiology 2005;81:446–51.
[12] Schenning APHJ, Feiters MC, Nolte RJM. An amphiphilic porphyrin with unexpected aggregation behaviour. Tetrahedron Letters 1993;34:7077–80.
[13] Kramer-Marek G, Serpa C, Szurko A, Widel M, Sochanik A, Snietura M, et al. Spectroscopic properties and photodynamic effects of new lipophilic porphyrin derivatives: efficacy, localization and cell death pathways. Journal of Photochemistry and Photobiology B: Biology 2006;84:1–14.
[14] Wilkinson F, Helman WP, Ross AB. Quantum fields for the photosensitized formation of the lowest electronically excited single state of molecular oxygen in solution. Journal of Physical and Chemical Reference Data 1993;22:113–262.
[15] Bonnett R, White RD, Winfield U-J, Berenbaum MC. Hydroporphyrins of the meso-tetra(hydroxyphenyl)porphyrin series as tumour photosensitizers. Biochemical Journal 1989;261:277–80.
[16] Rojkiewicz M, Zięba G, Jarczyk A, Kuś P. Lipophilicity of Tetraarylporphyrins. Part 1. Tetra-(Hydroxyphenyl)Porphyrins with long alkyl chain in the molecule. Journal of Planar Chromatography 2011;24:201–5.
[17] Nassar AEF, Amin M, Kamel AM, Clarimont C. Improving the decision-making process in the structural modification of drug candidates: enhancing metabolic stability. Drug Discovery Today 2004;9:1020–8.
[18] Lan M, Zhao H, Yuan H, Jiang C, Zuo S, Jiang Y. Absorption and EPR spectra of some porphyrins and metalloporphyrins. Dyes and Pigments 2007;74:357–62.
[19] Lovell JF, Liu TWB, Chen J, Zheng G. Activatable photosensitizers for imaging and therapy. Chemical Reviews 2010;110:2839–57.
[20] Ha JH, Ko S, Lee CH, Lee WY, Kim YR. Effect of core atom modification on photophysical properties and singlet oxygen generation efficiencies: tetraphenylporphyrin analogues core-modified by oxygen and/or sulfur. Chemical Physics Letters 2001;349:271–8.
[21] Monteiro CJP, Pereira MM, Azenha ME, Burrows HD, Serpa C, Arnaut LG, et al. A comparative study of water soluble 5,10,15,20-tetrakis(2,6-dichloro-3-sulfophenyl)porphyrin and its metal complexes as efficient sensitizers for photodegradation of phenols. Photochemical & Photobiological Sciences 2005;4:617–24.
[22] Silva EFF, Serpa C, Dąbrowski JM, Monteiro CJP, Formosinho SJ, Stochel G, et al. Mechanisms of singlet-oxygen and superoxide-ion generation by porphyrins and bacteriochlorins and their implications in photodynamic therapy. Chemistry - A European Journal 2010;16:9273–86.
[23] Mojzisowa H, Bonneau S, Maillard P, Berg K, Brault D. Photosensitizing properties of chlorins in solution and in membrane-mimicking systems. Photochemical & Photobiological Sciences 2009;8:778–87.
[24] Santosa V, Limantara L. Photodynamic therapy: new fight in medicine world. Indonesian Journal of Chemistry 2008;8:279–91.