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# Comparison of aggregation properties and photodynamic activity of phthalocyanines and azaphthalocyanines

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**Abstract**

Phthalocyanines (Pc) and their aza-analogues azaphthalocyanines (AzaPc) (tetrapyrazinoporphyrazines) with eight *n*-octylsulfanyl or *tert*-butylsulfanyl peripheral substituents and different central metals (Mg, Zn, metal-free) were synthesized. Dimerization constants $K_d$ and absorption spectra of pure monomeric and dimeric magnesium complexes in toluene were calculated using series of absorbances at different concentrations. The bulky *tert*-butylsulfanyl substituents were found to be much better inhibitors of aggregation than long alkyl chains. Also Pc are less aggregated in organic solvents then AzaPc, short explanation is given. Singlet oxygen production of Pc and AzaPc was compared using dye-sensitized photooxidation of 1,3-diphenylisobenzofuran in pyridine. Both Pc and AzaPc showed similar activity not dependent on type of peripheral substitution. Zinc complexes of both Pc and AzaPc exceeded the magnesium ones and metal-free dyes in singlet oxygen production approximately twice.


*Keywords:* Phthalocyanine; Azaphthalocyanine; Dimerization; Singlet oxygen; Aggregation

## 1. Introduction

Phthalocyanines (Pc) are well documented group of synthetic dyes that can be used as industrial dyes, optical sensors, in electrocatalytic oxidation, etc. Review on this topic has been published by Armstrong [1]. Photodynamic therapy (PDT) is one of the applications, where Pc have found their place [2–4]. This kind of cancer treatment is based on the combination of a photosensitizer (PS) and light. The PS absorbs light and is able, upon excitation, to transfer energy to surrounding molecules, including oxygen. Oxygen is then excited from the basic triplet state ($^3O_2$) to highly reactive singlet oxygen ($^1O_2$) that is mainly responsible for the cell death in PDT [5]. The high extinction coefficients of Pc and the Q-band bathochromic shift to approximately 670–700 nm make them a potentially suitable PS for PDT. Photosense (a mixture of sulfonated aluminum Pc, Fig. 1) is studied in Russia against breast [6] and head and neck cancer [7]. Pc4 (a silicon Pc, Fig. 1) was found to kill HIV viruses in red blood cells concentrates [8] and is used in some biological tests to produce PDT-mediated oxidative stress in cells [9,10]. Azaphthalocyanines (AzaPc) are analogues of Pc in which some carbons in benzene rings are replaced by nitrogens. AzaPc can be potentially used in applications similar to that of Pc. Recently, we have confirmed singlet oxygen production for different AzaPc. Alkylsulfanyl derivatives proved to be the most suitable candidates for PDT [11]. Photodynamic activity of AzaPc was found also by other group [12,13].

Aggregation is unfavorable property of Pc and AzaPc that decreases solubility and brings problems to purification and characterization. Moreover, aggregation shortens

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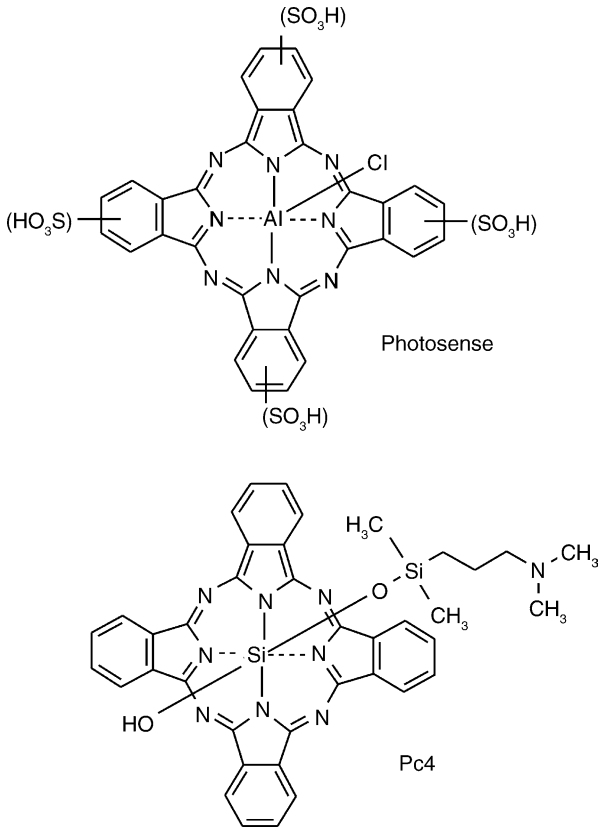



Fig. 1. Phthalocyanine photosensitzers. Photosense is a mixture of molecules with different number of sulfo groups.

the triplet-state lifetimes and reduces the singlet oxygen quantum yield. This behavior can be suppressed by introduction of long alkyl chains or bulky substituents to the periphery of the macrocycle [14,15]. However, it is not only the peripheral substitution that influences the level of the aggregation. Different solvents [16] (especially those able to coordinate central metal) and their concentration have a great influence on the aggregation behavior of the dye, too. The effect of various central metals was also investigated [17]. Central metals with more coordinating sites (Al, Si, Ge, Ga, Sn) [18] are favorable from this point of view, because they enable introduction of additional aggregation inhibiting substituent on the metal, and thus highly increase amount of monomer in solution or facilitate solubility at all [19]. Dissolving Pc in water environment strongly increases its dimer/monomer ratio, and the dyes are found practically only in the aggregated form. Several approaches were introduced to overwhelm this feature. One of them uses repulsion forces between ionized molecules of Pc. Introduction of eight anionic [20] or eight cationic [21] fully ionized moieties on the periphery resulted in a complete monomerization in water medium. The use of four cationic [22,23] or anionic [24] substituents suppresses the aggregation only partially, and complete monomerization is usually achieved with some additives, e.g. DMF [22], sodium dodecylsulfate [23], DMSO [24]. Lipophilic or amphiphilic Pc can be introduced to water using different delivery systems (unilamellar vesicles, ethoxylated plant oils, polysorbates, PEG, . . .) [25]. In such case, the peripheral substitution of Pc, its properties and concentration of solubilizers play an important role in disaggregation [26].

In the design of this work, we focused on the direct comparison of AzaPc and parent Pc. To the best of our knowledge, such work was not performed before. Introduction of nitrogen atoms to macrocyclic core can alter some properties of the Pc dye (aggregation, singlet oxygen production, solubility, UV–vis spectra, etc.). One of the scopes of this study was to find differences (if any) between these two types of macrocyclic systems disregarding the effect of peripheral substitution, central metal or solvent and to discuss shortly their suitability in photodynamic therapy.

As mentioned above, complete monomerization in water requires usage of strongly ionized molecules. Since it has been found [27,28] that amphiphilic compounds are more active than either hydrophilic or lipophilic PS, other strategies than introducing numerous charged substituents into the molecules of PS must be employed. The use of liposomes and unilamellar vesicles seems to be very advantageous in delivery of lipophilic or amphiphilic PS [25,29]. Dyes are usually incorporated in the lipid layer of the vesicle, and inhibition of aggregation is provided by peripheral substituents of Pc. For future investigations on photodynamic substances (e.g. amphiphilic), we needed to know whether it is better to use long alkyl chains or bulky substituents (standing for lipophilic part of molecule) to prevent aggregation.

We synthesized metal-free, magnesium(II) and zinc(II) complexes of Pc and AzaPc substituted with eight *tert*-butylsulfanyl (representing bulky substituents) or eight *n*-octylsulfanyl (representing long alkyl chain) substituents (**4**-**9**, **13**-**18**). We investigated their singlet oxygen production by means of a dye-sensitized photooxidation of 1,3-diphenylisobenzofuran (DPBF) in pyridine, where these dyes are not aggregated. Magnesium complexes were then chosen for monomer–dimer equilibrium studies in toluene which was found to be the best suited solvent for this part of study.

## 2. Experimental

All organic solvents used for the synthesis were of analytical grade. Anhydrous butanol and dioxane were stored over magnesium and sodium, respectively, and distilled prior to use. Pyridine was distilled prior to use in singlet oxygen measurements. 1,3-diphenylisobenzofuran, 2,2-dimethylpropane-1-thiol, octane-1-thiol and 4,5-dichloro-phthalonitrile were purchased from Aldrich, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was purchased from Fluka, copper(I) oxide was purchased from Lachema (Czech

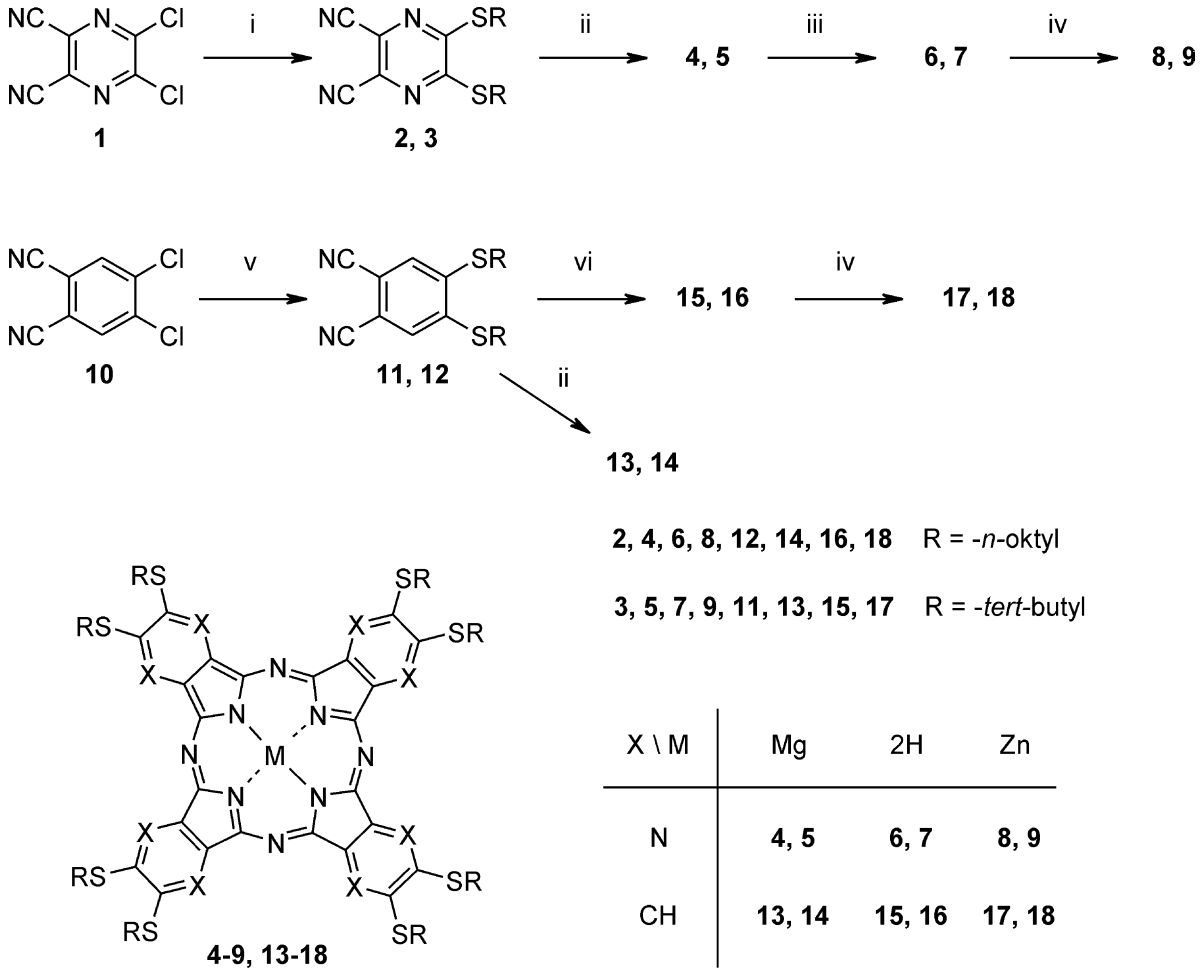
Republic). TLC was performed on Silufol UV 254 plates (Kavalier, Votice, Czech Republic). Merck Kieselgel 60 (0.040–0.063 mm) was used for column chromatography. Melting points were measured on Electrothermal IA9200 Series Digital Melting point Apparatus (Electrothermal Engineering Ltd., Southend-on-Sea, Essex, Great Britain) and are uncorrected. The elemental analysis was carried out on Automatic Microanalyser EA1110CE (Carlo Erba Instruments, S.p.A., Milano, Italy). Infrared spectra were measured in KBr pellets on IR-Spectrometer Nicolet Impact 400. $^{1}$H and $^{13}$C NMR spectra were recorded on Varian Mercury-Vx BB 300 (299.95 MHz, $^{1}$H; 75.43 MHz, $^{13}$C) Bruker Comp. (Karlsruhe, Germany). Chemical shifts reported are given relative to internal $Si(CH_3)_4$. UV–vis spectra were recorded on spectrophotometer UV-2401PC, Shimadzu Europa GmbH (Duisburg, Germany).

### 2.1. Synthesis

Synthesis (Scheme 1) of compounds **1**, **2** [30] and **4** [11] is described in literature. $^{1}$H NMR signals of dyes **5**, **7**, **8**, **9** and **13**-**18** showed strong broadening, aromatic $^{13}$C NMR signals of all AzaPc and Pc were poor, broad and very hard to detect.

#### 2.1.1. 5,6-Bis-tert-butylsulfanyl-pyrazine-2, 3-dicarbonitrile (**3**)

A solution of 1.85 g (20.6 mmol) of 2,2-dimethylpropane-1-thiol was stirred in 20.4 ml of aqueous NaOH (of concentration 1.0 M) at room temperature for 30 min. Then, a solution of 1.99 g (10.0 mmol) of **1** in 50 ml of tetrahydrofuran was added at once. The reaction mixture was stirred for another 30 min, evaporated, the residue dissolved in dichloromethane and washed several times with water. Organic phase was then dried ($Na_2SO_4$) and purified by chromatography on silica with toluene as eluent to yield 2.27 g (74%) of yellow needles, mp 161–162 °C (methanol). IR (KBr) 2964, 2924, 2903, 2233 (C≡N). $^{13}$C NMR ($CDCl_3$) $\delta$ 29.6, 52.3, 113.8, 125.0, 161.0. $^{1}$H NMR ($CDCl_3$) $\delta$ 1.64 (s, 9H, $CH_3$). Anal. found: C 54.62, H 5.89, N 18.02, S 20.66; Calc.: C 54.87, H 5.92, N 18.28, S 20.93.




Scheme 1. Reaction conditions: (i) HS-R, NaOH, THF/water; (ii) Mg, iodine, butanol; (iii) *p*-toluenesulfonic acid, THF/CHF; (iv) $Zn(COOCH_3)_2$, DMF/toluene; (v) HS-R, NaH, $Cu_2O$, DMF; (vi) DBU, butanol.

*2.1.2. {29H,31H-[2,3,9,10,16,17,23,24-octakis (tert-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza) phthalocyaninato](2-)-$N^{29}$,$N^{30}$,$N^{31}$,$N^{32}$} magnesium(II) (**5**)*

Absolute butanol was refluxed with 222 mg (9.1 mmol) of magnesium and a small crystal of iodine for 4 h, then 400 mg (1.3 mmol) of **3** was added and refluxing continued for next 3 h. Aqueous acetic acid (50% (v/v), 50 ml) was added after evaporation of the solvent, and the suspension was stirred for 30 min. Dark solid was filtered and washed with aqueous acetic acid, water and methanol. Crude **5** was absorbed to a small amount (2.5 g) of silica and washed with methanol on a glass frit until the solution was colorless. The product on silica was then dried up, poured on a silica column and purified by chromatography with chloroform/ethyl acetate (20:1) as eluent. The solvents were evaporated, and pure **5** was washed with methanol yielding 210 mg (51%) of fine dark green powder. $^{13}$C NMR ($CDCl_3$) $\delta$ 30.6, 51.2, 144.2, 151.1, 158.3. $^1$H NMR ($CDCl_3$) $\delta$ 1.94 (s, 72H, $CH_3$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 658 (298000), 596 (36400), 382 (145000).

*2.1.3. {29H,31H-[2,3,9,10,16,17,23,24-octakis (octylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza) phthalocyaninato](2-)-$N^{29}$,$N^{30}$,$N^{31}$,$N^{32}$}2H (**6**)*

Magnesium AzaPc **4** (600 mg, 0.35 mmol) was dissolved in 50 ml of chloroform and a solution of 670 mg (3.5 mmol) of *p*-toluenesulfonic acid in 20 ml of tetrahydrofuran was added. Mixture was stirred for 2 h at room temperature. After this time, solvent was evaporated and resulting solid was washed with water, hot methanol and hot acetone. Yield 452 mg (76%) of green powder. $^{13}$C NMR ($CDCl_3$) $\delta$ 14.1, 22.8, 28.7, 29.5, 29.6, 29.7, 31.8, 32.1, 142.5, 146.0, 158.7. $^1$H NMR ($CDCl_3$) $\delta$ 0.91 (t, 24H, $J$ = 6.8 Hz, $CH_3$), 1.27–1.62 (m, 64H, $CH_2$), 1.78 (p, 16H, $J$ = 6.9 Hz, $CH_2$), 2.12 (p, 16H, $J$ = 6.9 Hz, $CH_2$), 3.84 (t, 16H, $J$ = 7.1 Hz, S–$CH_2$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 655 (215800), 595 (26400), 385 (123000), (chloroform) 676 (211800), 643 (157800), 591 (29400), 487 (65400), 369 (157300).

*2.1.4. {29H,31H-[2,3,9,10,16,17,23,24-octakis (tert-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza) phthalocyaninato](2-)-$N^{29}$,$N^{30}$,$N^{31}$,$N^{32}$}2H (**7**)*

This compound was synthesized similar to compound **6** but starting from 180 mg (0.14 mmol) of **5** in 20 ml of chloroform and 270 mg (1.4 mmol) of *p*-toluenesulfonic acid in 10 ml of tetrahydrofuran. Yield 167 mg (95%) of green powder. $^{13}$C NMR ($CDCl_3$) $\delta$ 30.5, 51.6, 142.3, 146.5, 159.0. $^1$H NMR ($CDCl_3$) $\delta$ 2.19 (s, 72H, $CH_3$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 656 (228200), 595 (30400), 384 (128200), (chloroform), 675 (228700), 642 (172500), 590 (30500), 482 (65700), 366 (147900).

*2.1.5. {29H,31H-[2,3,9,10,16,17,23,24-octakis (octylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza) phthalocyaninato](2-)-$N^{29}$,$N^{30}$,$N^{31}$,$N^{32}$}zinc (II) (**8**)*

Two hundred and five milligrams (0.12 mmol) of metal-free AzaPc **6** was dissolved in 10 ml of toluene/*N*,*N*-dimethylformamide (DMF) (1:1) mixture, 267 mg (1.2 mmol) of zinc acetate was added, and the mixture was heated to 130 °C. Reaction was kept at this temperature for 2 h and cooled down. Excess of toluene was evaporated. Water (10 ml) was added to fine green suspension in rest of DMF, and the product filtered and washed thoroughly with water and methanol. Crude **8** was absorbed to a small amount of silica (1.5 g) and washed with methanol on a glass frit until the solution was colorless. The product on silica was then dried up, poured on silica column and purified by chromatography with chloroform/ethyl acetate (15:1) as eluent. The fractions containing pure **8** were evaporated to dryness. The residue was washed with methanol and acetone yielding 183 mg (86%) of dark green powder. $^{13}$C NMR ($CDCl_3$) $\delta$ 14.2, 22.8, 28.3, 29.3, 29.4, 29.6, 31.3, 32. 1, 143.7, 149.8, 157.4. $^1$H NMR ($CDCl_3$) $\delta$ 0.70–1.02 (m, 24H, $CH_3$), 1.02–1.56 (m, 80H, $CH_2$), 1.56–1.99 (m, 16H, $CH_2$), 2.43–3.81 (m, 16H, S–$CH_2$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 655 (297600), 594 (30400), 385 (145000).

*2.1.6. {29H,31H-[2,3,9,10,16,17,23,24-octakis (tert-butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza) phthalocyaninato](2-)-$N^{29}$,$N^{30}$,$N^{31}$,$N^{32}$}zinc (II) (**9**)*

Compound **9** was prepared following the procedure for **8** but starting from 100 mg (77 μmol) of **7** and 142 mg (0.77 mmol) of zinc acetate. Yield 83 mg (79%) of a green solid. $^{13}$C NMR ($CDCl_3$) $\delta$ 30.6, 51.2, 143.9, 150.6, 158.1. $^1$H NMR ($CDCl_3$) $\delta$ 1.90 (s, 72H, $CH_3$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 657 (298000), 595 (33600), 385 (146000).

*2.1.7. 4,5-Bis-tert-butylsulfanyl-phthalonitrile (**11**)*

Synthesis was performed in a manner similar to previously described method [14]. Thus 1.88 ml of 2,2-dimethylpropane-1-thiol (16.7 mmol) was slowly added into a suspension of 0.76 g of NaH (19 mmol) in anhydrous DMF (100 ml). After the end of $H_2$ development, 1.5 g of 4,5-dichlorophthalonitrile (**10**) (7.6 mmol) and 2.17 g of copper(I) oxide (15.2 mmol) were added, and the mixture was heated under stirring and inert gas at 90 °C for 30 min. The reaction was stirred at room temperature for additional 30 min and then poured into ice water (200 ml). The precipitate was filtered off, dissolved in chloroform and washed several times with water. Organic phase was purified by chromatography on silica with dichloromethane as eluent and recrystalized from methanol to yield 0.59 g (25%), mp 150–152 °C (methanol). IR (KBr) 3066, 2977, 2964, 2228 (C≡N). $^{13}$C NMR ($CDCl_3$) $\delta$ 146.9, 136.9, 115.2, 112.5, 49.6, 31.0. $^1$H NMR ($CDCl_3$) $\delta$ 7.26 (s, 2H, Ar–CH), 0.86 (s, 18H, $CH_3$).

*2.1.8. 4,5-Bis-octylsulfanyl-phthalonitrile (**12**)*

This compound was synthesized similar to compound **11** but starting from 1.94 ml of octane-1-thiol (11.2 mmol), 1.0 g of 4,5-dichlorophthalonitrile (**10**) (5.08 mmol), 0.51 g of NaH (12.7 mmol), 1.45 g of copper(I) oxide (10.2 mmol) and anhydrous DMF (50 ml). The crude product was purified by column chromatography on silica using dichloromethane as an eluent to give 1.76 g (83%) of off-white solid, mp 58 °C (methanol), IR (KBr) 3076 (arom. CH), 2957, 2924, 2853 (alif. CH), 2229 (C≡N). $^{13}$C NMR ($CDCl_3$) $\delta$ 144.2, 128.0, 115.7, 111.0, 32.7, 31.7, 29.1, 29.0, 28.9, 28.0, 22.6, 14.1. $^{1}$H NMR ($CDCl_3$) $\delta$ 7.44 (s, 2H, Ar–CH), 3.0 (t, 4H, $J$ = 7.6 Hz, $CH_2$–S), 1.75 (p, 4H, $J$ = 7.4 Hz, $CH_2$), 1.54–1.41 (m, 4H, $CH_2$), 1.37–1.22 (m, 16H, $CH_2$), 0.89 (t, 6H, $J$ = 6.5 Hz, $CH_3$).

*2.1.9. {29H,31H-[2,3,9,10,16,17,23,24-octakis (tert-butylsulfanyl)phthalocyaninato](2-)-$N^{29}$,$N^{30}$,$N^{31}$, $N^{32}$}magnesium(II) (**13**)*

Thirty nine milligrams of magnesium (1.6 mmol) and a small crystal of iodine were refluxed in anhydrous butanol (8 ml) for 4 h. Then 500 mg of 11 (1.6 mmol) was added, and the mixture heated under reflux for another 5 h. The precipitate was filtered, washed with methanol and acetone, dissolved in chloroform and purified by chromatography on silica with chloroform and later with hexane/ethyl acetate (1:1, 1:2) as eluent to yield 156 mg (31%) of dark green solid. $^{13}$C NMR ($CDCl_3$) $\delta$ 137.5, 130.5, 128.3, 124.7, 48.6, 31.4. $^{1}$H NMR ($CDCl_3$) $\delta$ 7.97 (s, 8H, Ar–H), 1.45 (s, 72H, $CH_3$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 705 (275000), 636 (48800), 371 (93400).

*2.1.10. {29H,31H-[2,3,9,10,16,17,23,24-octakis (octylsulfanyl)phthalocyaninato](2-)-$N^{29}$,$N^{30}$,$N^{31}$, $N^{32}$}magnesium(II) (**14**)*

This compound was prepared by the procedure described for **13** but from 20 mg of magnesium (0.8 mmol) anhydrous butanol (10 ml) and 300 mg of **12** (0.72 mmol). After cooling, precipitate was purified by chromatography on silica with chloroform to yield 125 mg (41%). $^{13}$C NMR ($CDCl_3$) $\delta$ 139.8, 132.6, 128.1, 125.5, 34.0, 31.9, 29.4, 29.2, 29.1, 28.6, 22.7, 14.2. $^{1}$H NMR ($CDCl_3$) $\delta$ 8.11–8.07(bs, 8H, Ar–H), 3.66–3.48 (m, 16H, $CH_2$–S), 2.15–1.90 (m, 32H, $CH_2$), 1.85–1.15 (m, 64H, $CH_2$), 1.10–0.80 (m, 24H, $CH_3$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 711 (346900), 639 (59600), 376 (114800).

*2.1.11. {29H,31H-[2,3,9,10,16,17,23,24-octakis (tert-butylsulfanyl)phthalocyaninato](2-)-$N^{29}$,$N^{30}$,$N^{31}$, $N^{32}$}2H (**15**)*

Compound **11** (2.2 g, 7.2 mmol) was heated in anhydrous butanol (100 ml) in the presence of DBU (1.25 ml) under stirring and reflux for 4 h. After disappearance of starting material the mixture was cooled down, the solvent was evaporated, the rest was washed with methanol and acetone and then purified by chromatography on silica with chloroform as eluent to yield 300 mg (14%) of dark green solid. $^{13}$C NMR ($CDCl_3$) $\delta$ 139.0, 130.0, 128.2, 125.5, 48.9, 31.4. $^{1}$H NMR ($CDCl_3$) $\delta$ 8.10 (s, 8H, Ar–H), 1.43 (s, 72H, $-CH_3$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 719 (119500), 695 (119100), 636 (29000), 356 (64700).

*2.1.12. {29H,31H-[2,3,9,10,16,17,23,24-octakis (octylsulfanyl)phthalocyaninato](2-)-$N^{29}$,$N^{30}$,$N^{31}$, $N^{32}$}2H (**16**)*

This compound was prepared by the procedure described for **15** but from 2.2 g of 12 (5.3 mmol), 1.25 ml of DBU and anhydrous butanol (100 ml). Yield 305 mg (14%). $^{13}$C NMR ($CDCl_3$) $\delta$ 139.7, 132.5, 128.2, 125.5, 34.0, 32.1, 29.7, 29.6, 29.5, 28.9, 22.8, 14.2. $^{1}$H NMR ($CDCl_3$) $\delta$ 8.29 (s, 8H, Ar–H), 3.39 (t, 16H, $J$ = 7.1 Hz, $CH_2$–S), 2.15–2.0 (m, 16H, $CH_2$), 1.85–1.70 (m, 16H, $CH_2$), 1.62–1.32 (m, 64H, $CH_2$), 0.93 (t, 24H, $J$ = 6.5 Hz, $CH_3$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 733 (115000), 707 (140200), 638 (21600), 366 (40000).

*2.1.13. {29H,31H-[2,3,9,10,16,17,23,24-octakis (tert-butylsulfanyl)phthalocyaninato](2-)-$N^{29}$,$N^{30}$, $N^{31}$,$N^{32}$}zinc(II) (**17**)*

Compound **15** (60 mg, 0.05 mmol) was dissolved in 3 ml of anhydrous DMF and toluene (2:1) mixture. Anhydrous zinc acetate (91 mg, 0.5 mmol) was added and the mixture was stirred at 120 °C for 1 h. After cooling, the solvents were evaporated, the dark solid was dissolved in chloroform and chromatographed on silica with chloroform as eluent to yield 51 mg (81%) of dark-green solid. $^{13}$C NMR ($CDCl_3$) $\delta$ 135.7, 133.2, 130.1, 125.5, 48.6, 31.5. $^{1}$H NMR ($CDCl_3$) $\delta$ 6.98 (s, 8H, Ar–H), 1.43 (s, 72H, $CH_3$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 692 (273400), 625 (44700), 360 (83500).

*2.1.14. {29H,31H-[2,3,9,10,16,17,23,24-octakis (octylsulfanyl)phthalocyaninato](2-)-$N^{29}$,$N^{30}$,$N^{31}$, $N^{32}$}zinc(II) (**18**)*

This compound was obtained from 83 mg of 16 (0.05 mmol), 91 mg of zinc acetate (0.5 mmol) in a mixture of anhydrous solvents by a method reported in preparation of 17. Yield is 62 mg (72%). $^{13}$C NMR ($CDCl_3$) $\delta$ 139.5, 132.3, 128.1, 125.2, 33.7, 32.0, 29.5, 29.4, 29.3, 28.6, 22.8, 14.1. $^{1}$H NMR ($CDCl_3$) $\delta$ 8.10–7.80 (bs, 8H, Ar–H); 3.13–2.74 (bs, 16H, $CH_2$–S), 2.00–1.63 (bs, 32H, $CH_2$), 1.60–1.20 (bs, 64H, $CH_2$), 1.00–0.85 (bs, 24H, $CH_3$). UV–vis (pyridine) $\lambda_{max}$ ($\varepsilon$) 712 (278800), 638 (47400), 376 (79300).

*2.2. Singlet oxygen production measurements*

Singlet oxygen measurements were carried out by 1,3-diphenylisobenzofuran (DPBF) decomposition reaction. Dyes ($5.0 \times 10^{-6}$ M) and DPBF ($50.0 \times 10^{-6}$ M) were dissolved in pyridine, transferred to a glass tube in the dark and irradiated from a distance 0.5 m for different times under vigorous stirring. A halogen lamp (Tip, 200 W) was used as a light source. Light under 550 nm was filtered off using orange

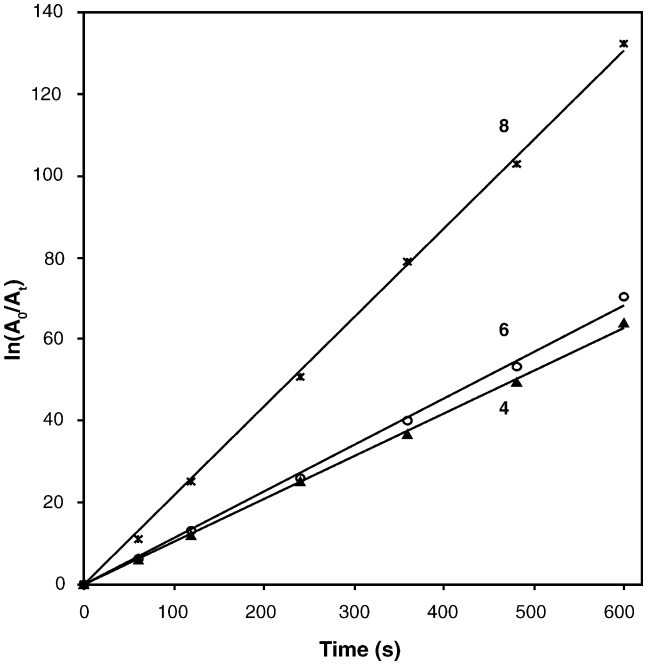



Fig. 2. Time-dependent decomposition of DPBF by singlet oxygen produced by magnesium (**4**), metal-free (**6**) and zinc (**8**) AzaPc.

HOYA G filter to remove self-decomposition of DPBF. The decomposition of DPBF was measured as a decrease of absorbance at 417 nm.

Singlet oxygen production is generally given as a singlet oxygen quantum yield ($\Phi_\Delta$). It is defined as the number of $^1O_2$ molecules formed per absorbed photon. One of the methods for determination of $\Phi_\Delta$ uses a standard dye of a known singlet oxygen quantum yield ($\Phi_{\Delta\text{standard}}$) and slopes of the plot of time-dependent decomposition of a chemical scavenger of $^1O_2$, e.g. DPBF [31]. The singlet oxygen quantum yield of the sample ($\Phi_{\Delta\text{sample}}$) is then calculated (Eq. (1)):

$$\Phi_{\Delta\text{sample}} = \Phi_{\Delta\text{standard}} k_{\text{standard}} / k_{\text{sample}} \quad (1)$$

where $k_{\text{standard}}$ and $k_{\text{sample}}$ are the slopes of the plot of the time-dependent decrease of DPBF, expressed as the decrease of absorbance $A$ at 417 nm ($d(\ln(A_0/A_t))/d(t)$) (Fig. 2) of the standard dye and the measured sample, respectively. Since we could not find a suitable standard measured under the same conditions (pyridine, light over 550 nm), we compared only the slopes and not $\Phi_\Delta$. However, for our purpose of comparing the compounds one to another, it was satisfactory. Because the compounds have not exactly the same UV–vis spectrum and, consequently, also amount of absorbed light is not the same, we related the slopes to integrated area under absorption spectrum over 550 nm.

### 2.3. Monomer–dimer equilibrium

UV–vis spectra of the chosen samples were recorded in toluene at different concentrations ranging from $10^{-5}$ to $10^{-8}$ M using a 1 cm quartz cell. Samples (especially those of Pc) were stored in the dark and measured as soon as possible after dilution. Pc and AzaPc are known to exist in solutions in the form of monomers, dimers, trimers and oligomers. However, in such dilute solutions (under $10^{-4}$) no higher aggregates than dimers are believed to be present [32]. In this case, the dimerization constant ($K_d$) of monomer–dimer equilibrium is:

$$K_d = [d]/[m]^2 \quad (2)$$

total concentration ($C_t$):

$$C_t = 2[d] + [m] \quad (3)$$

and absorbance ($A$) measured at concentration $C_t$:

$$A = \varepsilon_m[m] + 2\varepsilon_d[d] \quad (4)$$

Combining and rearranging Eqs. (2)–(4) we get:

$$A = \varepsilon_d C_t + (\varepsilon_m - \varepsilon_d)[-1 + \sqrt{(1 + 8K_d C_t)}]/4K_d \quad (5)$$

where $[m]$ and $[d]$ are concentrations of monomer and dimer, respectively, $\varepsilon_m$ is extinction coefficient ($M^{-1}cm^{-1}$) of monomer at given wavelength, $\varepsilon_d$ the extinction coefficient ($M^{-1}cm^{-1}$) of one Pc or AzaPc molecule of dimer at given wavelength. In the Eq. (5), $A$ and $C_t$ are measurable quantities and parameters $\varepsilon_m$, $\varepsilon_d$ and $K_d$ were determined by nonlinear regression using the series of concentrations and corresponding absorbances at maximum of the Q-band of monomer of each sample measured. Nonlinear regression was computed in Microsoft® Office Excel 2003 environment with the use of Solver add-in. Determination of the three unknown parameters with a satisfactory precision required not only sufficient amount of data (in our case 10 concentrations) but also sufficient extent of values of the concentrations and corresponding absorbances. This was not completely fulfilled in the case of **4** and **13**. That is why, some of their data had to be taken exactly from the absorption spectra. The best fitted value of the $K_d$ was then fixed, and this procedure repeated for different wavelengths to obtain absorption spectra of pure monomer and dimer (Figs. 3 and 4).

## 3. Results and discussion

### 3.1. Singlet oxygen production measurements

Results are summarized in Table 1 (AzaPc) and Table 2 (Pc). Data in the tables are relative and serve only for comparison of these twelve compounds. Measurements of the

Table 1
Values of the slopes $k$ of the plot of time-dependent dye-sensitized decomposition of DPBF (for AzaPc)

| | Mg | 2H | Zn |
|---|---|---|---|
| *n*-Octyl | 19.3 (**4**) | 19.9 (**6**) | 37.3 (**8**) |
| *tert*-Butyl | 18.2 (**5**) | 20.8 (**7**) | 37.6 (**9**) |

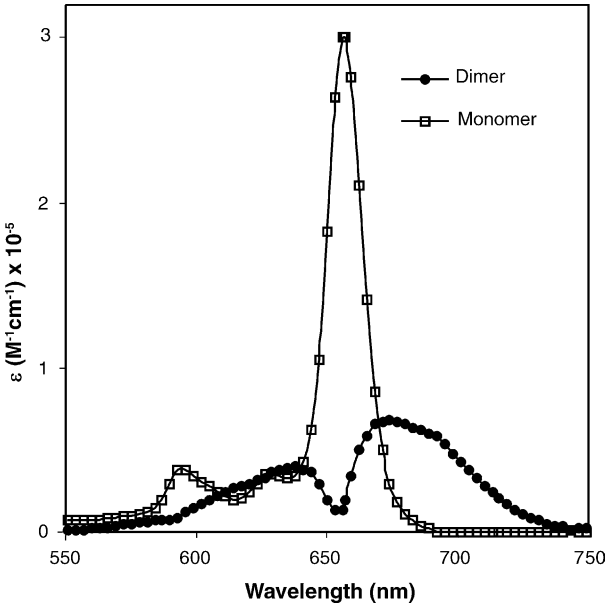



Fig. 3. Calculated absorption spectra of pure monomeric (□), and dimeric (●) AzaPc **5**.

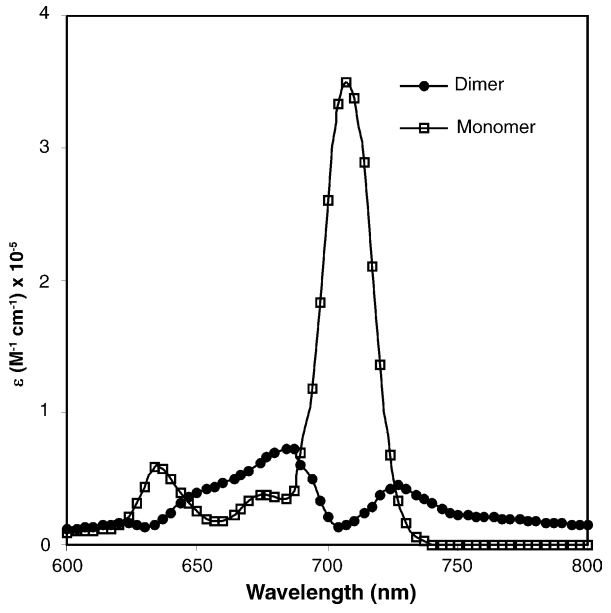



Fig. 4. Calculated absorption spectra of pure monomeric(□), and dimeric (●) Pc **14**.

singlet oxygen were performed in pyridine, where the aggregation to dimers is negligible and can be disregarded. The UV–vis spectra of all compounds in pyridine were sharp, the extinction coefficients in area of the Q-band were not rising further with dilution, and their values for Mg complexes reached values very close to those counted later for monomer (see Section 3.2) thus suggesting that only monomers were present in solution. No changes of the spectra in the area of the Q-band were found, even after 10 min of illumination, thus suggesting that no decomposition of the dyes took place. It is in contrast to our later findings for Pc in toluene during experiments with monomer–dimer equilibrium (see Section 3.2). Surprisingly, spectra of metal-free AzaPc **6** and **7** in pyridine did not show $D_{2H}$-type splitting of Q-band observed in other solvents (tetrahydrofuran, chloroform and toluene).

Table 2
Values of the slopes *k* of the plot of time-dependent dye-sensitized decomposition of DPBF (for Pc)

| | Mg | 2H | Zn |
|---|---|---|---|
| *n*-Octyl | 20.0 (**14**) | 17.4 (**16**) | 42.6 (**18**) |
| *tert*-Butyl | 18.7 (**13**) | 18.2 (**15**) | 39.3 (**17**) |

As expected, no marked difference was observed regarding the influence of the used peripheral substituents on singlet oxygen production. The more surprising is comparable activity of Mg and metal-free compounds. Mg complexes were formerly found to be much more potent than the metal-free compounds [11,33,34]. At least in the case of AzaPc it can be concerned with no splitting of metal-free dyes spectra, but we do not have any sufficient explanation. Moreover, metal-free Pc spectra are split in Q-band, so it cannot be generalized. Zinc complexes of both Pc and AzaPc exceeded the former ones almost twice in activity, so the hypothesis that they belong to the best from the point of view of $^1O_2$ production has been again confirmed [34]. Comparing the activity of an azaanalogue and a parent Pc macrocyclic system we did not find any significant difference. Focusing on the zinc derivatives, Pc can be considered a little bit stronger $^1O_2$ producers, however, this difference is very small.

### 3.2. Monomer–dimer equilibrium

Since both *n*-oktylsulfanyl and *tert*-butylsulfanyl substituents readily inhibit aggregation, the difference between them can not be well observed in solvents like pyridine, chloroform, dichloromethane or tetrahydrofuran in which Pc and AzaPc are present mainly in monomer form even at higher concentrations ($5.0 \times 10^{-5}$ M). Therefore, we were searching for a solvent with higher variation in aggregation properties among studied compounds. Finally toluene was found to suit best for this experiment. Nevertheless, even this solvent was not ideal. The variance in aggregation properties of the compounds studied was too great as it can be seen on dependence of $\varepsilon_{max}$ at Q-band of monomer on concentration (Fig. 5), but it was the best solvent among all we tried. That is why some data could not be counted and were taken exactly from the UV–vis spectra.

Some spectroscopic data are given in the Table 3.

Data for compound **4** could not be completely analyzed because of very strong aggregation behavior (as obvious also from very high $K_d$ exceeding the other compounds in orders). This compound was very strongly aggregated in toluene even at concentration $1.0 \times 10^{-8}$ M that was a detection limit (even when we used 10 cm cell) of our instrument. Such obtained data were satisfactory to count $K_d$ and $\varepsilon_{655}$ (at $\lambda_{max}$ of the Q-band of monomer) but not for counting the whole spectra of pure monomer and dimer. The spectrum of compound **4**

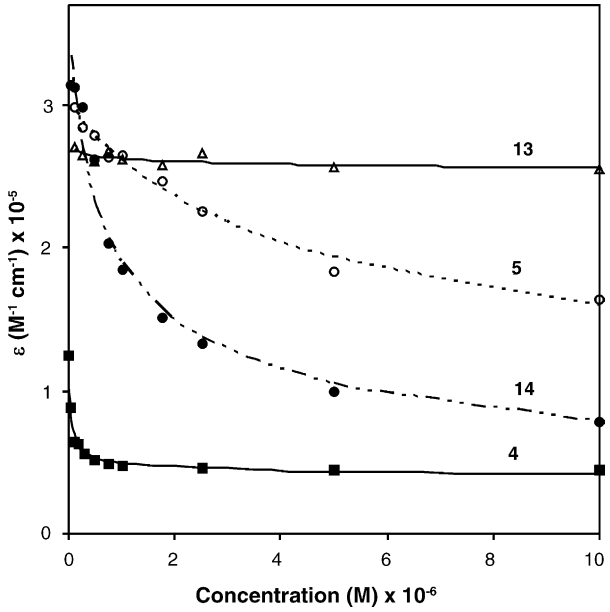



Fig. 5. Experimental (points) and calculated (lines) plots of extinction coefficient vs. concentration for: **4** (■), **5** (◯), **13** (△), and **14** (●).

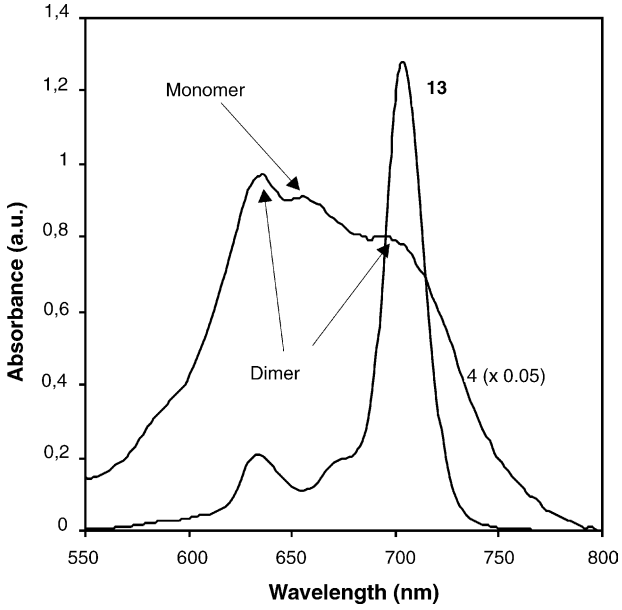



Fig. 6. Absorption spectra of **4** ($1.0 \times 10^{-6}$ M) and **13** ($5.0 \times 10^{-6}$ M) in toluene with marked $\lambda_{max}$ of monomer (655 nm) and dimer (634 and 700 nm) of **4**.

from which $\lambda_{max}$ of monomer and dimer were taken is presented in Fig. 6. In this figure, the spectrum of the dimer of *n*-octylsulfanyl AzaPc (**4**) is expected to be similar rather to *n*-octylsulfanyl Pc (**14**) (higher $\varepsilon_{max}$ of the first dimer band) than to *tert*-butylsulfanyl AzaPc (**5**). Hence, the shape of the dimer spectrum is dependent rather on the nature of the peripheral chain than on type of macrocyclic system.

On the other hand, spectrum of concentrated **13** (Fig. 6) is very similar to spectrum of pure monomer of **14** (Fig. 4), hence it follows that Pc **13** is almost fully disaggregated at all concentrations measured. For this reason, its $K_d$ is expected to be much lower than for other samples (perhaps even several orders) and can not be counted using the nonlinear regression at this range of concentrations. $\lambda_{max}$ and $\varepsilon_{max}$ for monomer of **13** were obtained directly from the absorption spectrum (at low concentration $1.0 \times 10^{-7}$ M) without any calculation.

Comparing the influence of different substituents on aggregation properties of the studied compounds, *n*-octylsulfanyl contribution to monomerization is much smaller (in range of orders) than that of bulky *tert*-butylsulfanyl. These results show the bulky substituents being more suitable for suppression of dimerization than long alkyl chains.

Great differences were found also between AzaPc and Pc. It seems the replacement of carbon atom in benzene ring by nitrogen leads to stronger aggregation. One of the proposed explanations is that the density of π-electrons on the nitrogen atoms is increased due to higher electronegativity resulting in increased electrostatic interactions between two molecules of the dye. Similar results were found previously for naphthalocyanines and their aza-analogues tetraquinoxalinoporphyrazines [35]. These observations contradict the predictions of some authors [36,37] who synthesized the AzaPc in a good belief in better solubility and less aggregation.

Table 3
Summary of aggregation and spectroscopic data

| | AzaPc | | Pc | |
|---|---|---|---|---|
| | **4** | **5** | **13** | **14** |
| Monomer | | | | |
| $K_d$ ($M^{-1}$) | $3.52 \times 10^8$ | $9.62 \times 10^4$ | n/a | $7.82 \times 10^5$ |
| $\lambda_{max}$ (nm) | 655 | 656 | 702[a] | 707 |
| $\varepsilon_{max} \times 10^5$ ($M^{-1}cm^{-1}$) | 3.16 | 3.01 | 2.80[a] | 3.50 |
| Dimer | | | | |
| $\lambda_{max}$ (nm) | 634[a], 700[a] | 638, 674 | n/a | 687, 727 |
| $\varepsilon_{max} \times 10^{4b}$ ($M^{-1}cm^{-1}$) | n/a | 3.98, 6.77 | n/a | 7.33, 4.54 |

n/a, not analyzed.
[a] Data obtained exactly from absorption spectrum.
[b] Values are given as $\varepsilon_{max}$ of one molecule of dimer and correspond to values $\lambda_{max}$ in the line above.

We observed the unusually strong decomposition of Pc samples (approximately 30–40% in 4 h) at room light during the experiments in toluene. This can be attributed to true photobleaching of the macrocyclic core since we observed decrease throughout all the Pc absorption spectra [38]. That is why, these samples were stored strictly in the dark and data were taken as soon as possible after diluting. Such behavior was not found for AzaPc samples suggesting the AzaPc core is more stable.

## 4. Conclusions

Both substituents (*n*-oktylsulfanyl and *tert*-butylsulfanyl) serve as good inhibitors of aggregation as can be observed in solutions of well-coordinating solvents (pyridine, tetrahydrofuran) where such derived Pc and AzaPc stay in the form of monomers even at high concentrations. Bulky substituents, however, provide much better monomerization in solvents where differences can be observed (toluene). Since it will be necessary to ensure the best disaggregation properties in liposomes or unilamellar vesicles, the use of *tert*-butylsulfanyl substituets seems to be more advantageous.

Comparing the properties of only macrocyclic system we found Pc to be better in some parameters. Compounds with the same peripheral substitution, central metal and in the same solvent were compared to one another to eliminate any differences except the different macrocyclic system. The ability of Pc for aggregation is much lower as can be seen from $K_d$ values for similar compounds ($3.52 \times 10^8\,M^{-1}$ and $7.82 \times 10^5\,M^{-1}$ for *n*-octylsulfanyl AzaPc **4** and Pc **14**, respectively, and $9.62 \times 10^4\,M^{-1}$ and very low—undetectable in this experiment—for *tert*-butylsulfanyl AzaPc **5** and Pc **13**, respectively). Bathochromic shift of the Q-band of Pc by almost 50 nm compared to AzaPc is also a great advantage in PDT where every 10 nm increase strongly the depth of photodynamic intervention. On the other hand, AzaPc and Pc can be considered almost equal in $^1O_2$ production that makes AzaPc possible PS for purposes where the light penetration depth is not so important, e.g. skin non-melanotic diseases. The disadvantage of Pc is their fast photobleaching observed in toluene. Such handicap strongly limits their use. However, this behavior was observed only in toluene (e.g. not in pyridine even during strong illumination in singlet oxygen experiments) and will need to be more investigated. AzaPc are, from this point of view, much more stable.

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