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Synthesis and comparison of photodynamic activity of alkylheteroatom substituted azaphthalocyanines

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Abstract

Optimal reaction conditions were developed for synthesis of octakis(butylamino), octakis(butylsulfanyl) and octakis(butoxy) azaphthalocyanines (AzaPc's) with central metal Mg, Zn and metal-free. Their photodynamic activity was measured and compared as a dye-sensitised photooxidation of 1,3-diphenylisobenzofurane (DPBF). Compounds with alkylamino substituent are very poor producers of the singlet oxygen and therefore not suitable as sensitizers for photodynamic therapy (PDT). On the other hand, compounds with alkylsulfanyl and alkoxy substituents possess very good photodynamic activity and are suitable for PDT.

Keywords: Azaphthalocyanines; Tetrapyrazinoporphyrazines; Photodynamic therapy; Singlet oxygen

1. Introduction

Tetrapyrazinoporphyrazines, a group of azaphthalocyanines (AzaPc's), are aza-analogues of synthetic dyes phthalocyanines (Pc's). As they differ from them only in the presence of eight more nitrogen in the structure of basic core, they could be used in very similar applications. For more information about the use of Pc's as materials see the review of Armstrong [1]. Photodynamic therapy (PDT) is one of the applications where Pc's has found their place in recent years, too. It is a medical treatment of cancer (but not only) that employs the combination of light and photosensitizer (e.g. Pc's) [2]. Photosensitizer is getting excited after the absorption of light of proper wavelength and can undergo an intersystem crossing to its triplet state. Releasing from this state to a ground state involves several mechanisms, e.g. phosphorescence or energy transfer to oxygen [3]. The last mentioned mechanism is of crucial importance for PDT since arising singlet oxygen ($^1O_2$) is the main active species in this process [4,5].

Not so long ago, we investigated a new group of potential photosensitizers from the group of water-soluble alkylamino AzaPc's [6]. We have not found any activity on cells in vitro. However, they produced singlet oxygen as it was confirmed by a dye-sensitised photooxidation of 1,3-diphenylisobenzofurane (DPBF). During some other experiments, we prepared alkoxy AzaPc's and contrary to alkylamino derivatives they possess a very intense fluorescence. Some authors [7] consider the ability of fluorescence to be connected with the ability of singlet oxygen production. Therefore, we decided to prepare the series of alkylheteroatom substituted AzaPc's and to investigate and compare their $^1O_2$ production. We chose four-carbon chains in a belief that it is long enough to inhibit aggregation. Aggregation to dimers and "stocking", that is unfavourable property of all Pc's and AzaPc's and which brings a lot of problems during purification and characterization of prepared compounds, can be suppressed by introduction of either bulky or long chains to periphery of macrocycle [8].

Activity of Pc's (and therefore perhaps also AzaPc's) strongly depends on the present central metal, e.g. Zn (the best one), Al and Mg complexes which are suitable for PDT [9,10]. On the other hand, metal-free or e.g. Co, Ni or Cu Pc's are very weak producers of the singlet oxygen [10]. That is why the Zn complexes were chosen as the final products.

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2. Results and discussion

2.1. Synthesis of the precursors

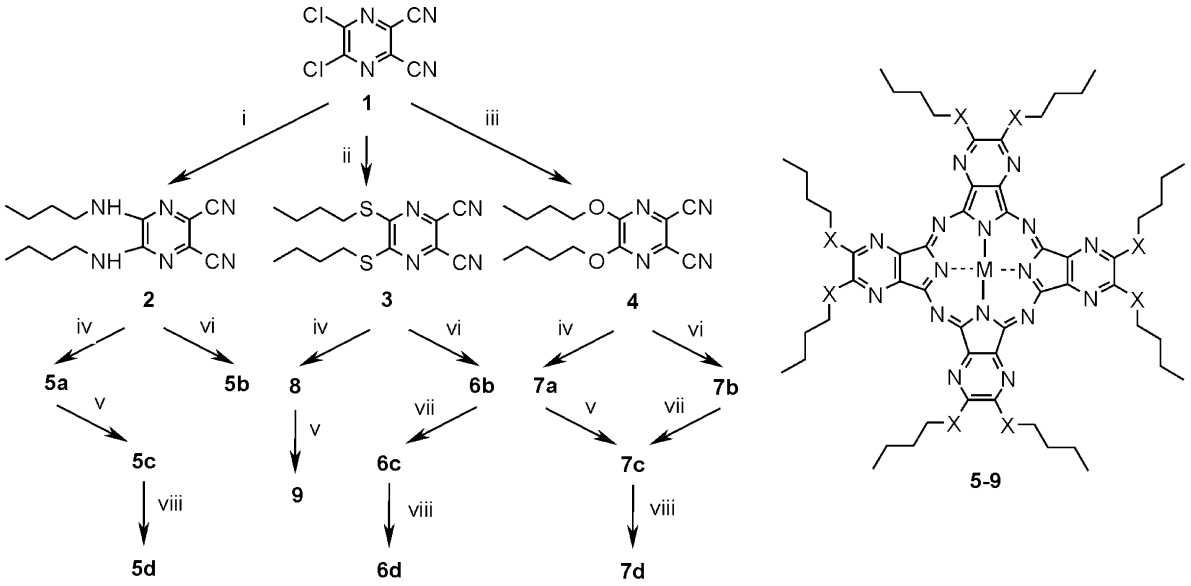
As a starting material was chosen 5,6-dichloro-pyrazine-2,3-dicarbonitrile (**1**) that is easily accessible from not very expensive diaminomaleodinitrile in a two step reaction [11,12]. This process involves condensation with highly reactive oxalylchloride to 2,3-dioxo-1,2,3,4-tetrahydropyrazine-5,6-dicarbonitrile and then, in the second step, halogenation of this compound with thionyl chloride to produce the desired product **1**.

Because of the strong electron withdrawing ability of pyrazine-2,3-dicarbonitrile moiety and chlorine atoms, this compound undergoes ready substitution in positions 5 and 6 with nucleophiles like primary [12,13] and secondary amines [14], alcoholates [15], thiolates [11]. Consequently, treating **1** with butyl-1-amine at room temperature (r.t.) afforded **2** in good yields. Also **3** can be prepared at r.t. in reaction of butane-1-thiol with **1**. Either pyridine [11] or aqueous NaOH can be used for generation of thiolate. Reaction with pyridine required approximately 12 h to achieve **3**. On the other hand, reaction with thiolate produced by aq. NaOH ran very fast and was finished almost immediately. Therefore, the latter method was chosen for the next synthesis. Due to low acidity of alcohols compared to thiols, it is necessary to keep anhydrous conditions during preparation of **4**. A lot of side reactions took place on cyano groups when using sodium for butanolate preparation, so we employed a described method using triethylamine [15] (Scheme 1).

2.2. Synthesis of azaphthalocyanines

There are lots of procedures introduced for cyclization of starting derivatives of pyrazine-2,3-dicarbonitrile, e.g. the use of magnesium [15,16] or lithium alcoholates [17], 1,8-diazabicyclo[5,4,0]-7-undecene (DBU) [18], urea and appropriate metal salt in formamide [15] or quinoline [16] and also conversion of dicarbonitrile to highly reactive isoindolinediimines [14]. Zinc AzaPc can also be readily prepared from metal-free AzaPc by reaction with zinc acetate in dimethylformamide. Metal-free AzaPc can be obtained from either dilithium or magnesium AzaPc using weak or strong acid, respectively.

The most widespread procedure of obtaining dilithium or magnesium AzaPc's is the use of alcoholates. However,



X= \ M=	2Li	Mg	2H	Zn
NH	**5a**	**5b**	**5c**	**5d**
S		**6b**	**6c**	**6d**
O	**7a**	**7b**	**7c**	**7d**
S/O	**8**		**9**	

i) $C_4H_9NH_2$, THF
ii) C_4H_9SH, NaOH, THF/water
iii) butanol, TEA
iv) butanol, Li
v) acetic acid, water
vi) butanol, Mg
vii) p-toluenesulphonic acid, DCM/THF
viii) $Zn(CH_3COO)_2$, DMF/toluene)

Scheme 1.

even for this method there are big differences in reaction time varying for similar reactions from 15 min [16] through overnight period [11] to even 92 h [15]. Because of such a big variance of reaction conditions we decided to compare the reactivity of magnesium and lithium alcoholates and search for the optimal reaction time for cyclotetramerization of dinitriles **2**–**4**. The method for this measurement is described in Section 4.2. A great advantage of this method is the specific absorption of arising AzaPc's in the Q band region (about 600–800 nm) and that is why the yield can be determined as a functionality of the absorbance at λ_{max} of each AzaPc **5**–**7**. Neither starting materials, reaction intermediates nor side products have any absorption in this region and therefore no interference occurs.

Dilithium AzaPc's **5a** and **7a** are labile against mild acidic conditions and even water is strong enough to demetalate them to various extent into metal-free AzaPc (this causes changes in the UV–Vis spectrum and disables the right reproduction of the results). We could not be sure that all AzaPc stayed in the dilithium form during working up the taken samples and ratio of metal-free and dilithium compounds was not predictable. That is why, whole compounds **5a** and **7a** were converted to **5c** and **7c** before each measurement of absorbance, by addition of small amount of acetic acid. Reasons for missing data for the metal-free octakis(butylsulfanyl) AzaPc **6c** are discussed below.

Results of this study are summarized into graphs. As evident, the compounds with oxygen (both **7b** and **7c**) were unstable and started to decompose after they reached maximum yield in approximately 3 h (Figs. 1 and 2). Such decomposition was observed neither for nitrogen (**5b**, **5c**) nor sulphur (**6b**) compounds. When comparing yields of these two methods (using Mg or Li), the magnesium AzaPc's **6b** and **7b** reach unusually high results. However, the real yields are much lower due to big losses during purification. On the other hand, we were surprised by a very low reaction speed for magnesium AzaPc **5b** reaching the yield only about 3% in 1 h (Fig. 3). The maximum yield of **5b** reached the same

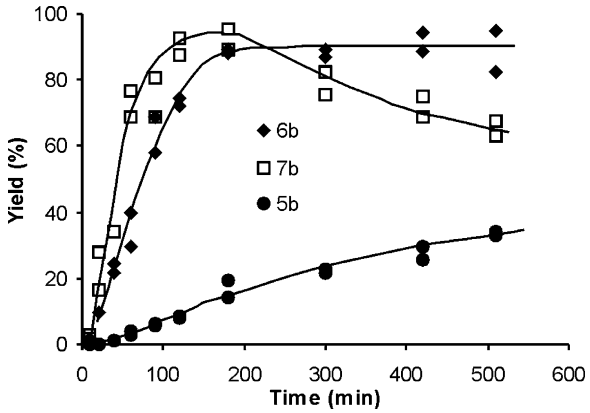


Fig. 2. Dependence of the AzaPc's yield on the reaction time. Reaction with Mg butanolate.

value as for **5c** after approximately 24 h and did not rise anymore.

The stability of peripheral chain connection under basic conditions produced by alcoholates was another problem during synthesis. There were found no problems with nitrogen connection. That matches our former findings [6]. Stability of oxygen connection was considered by Mørkved et al. [15]. They found that side chains connected to AzaPc core through oxygen are not stable and are usually replaced to some extent by the alcoholate used for cyclization (even for the magnesium alcoholate). In our case, it was not a problem because we chose butoxy substituent and lithium or magnesium butanolate for cyclization. The problems started with sulphur connection. When using lithium butanolate, we found that most of the peripheral butylsulfanyl substituents have been replaced by butoxy moiety. ^{1}H NMR of **9** shows that ratio of these substituents, given as ratio of the hydrogens adjacent to heteroatom (δ 5.24–4.84 (m) for O–CH_2 and δ 3.90–3.58 (m) for S–CH_2), is approximately 3:1 (butoxy/butylsulfanyl) after the reaction time of 3 h. The position and amount of butoxy substituents per each single molecule is random and unpredictable. The replacement can also be traced in the UV–Vis spectrum (Fig. 4). Pure

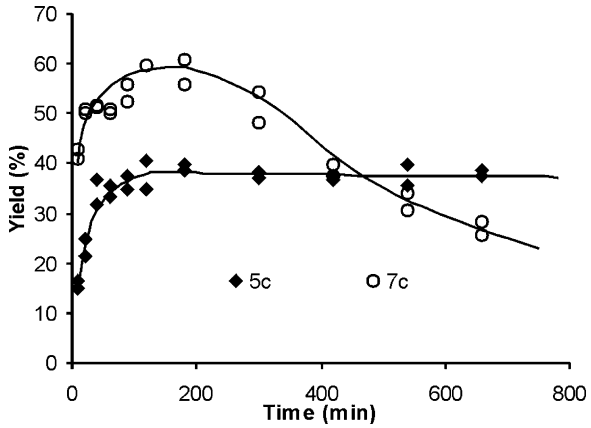


Fig. 1. Dependence of the AzaPc's yield on the reaction time. Reaction with Li butanolate.

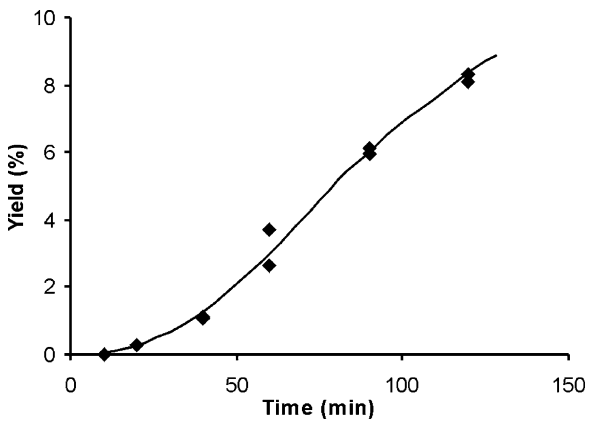


Fig. 3. Dependence of the yield of **5b** on the reaction time. Reaction with Mg butanolate (times up to 150 min—part of the Fig. 2).

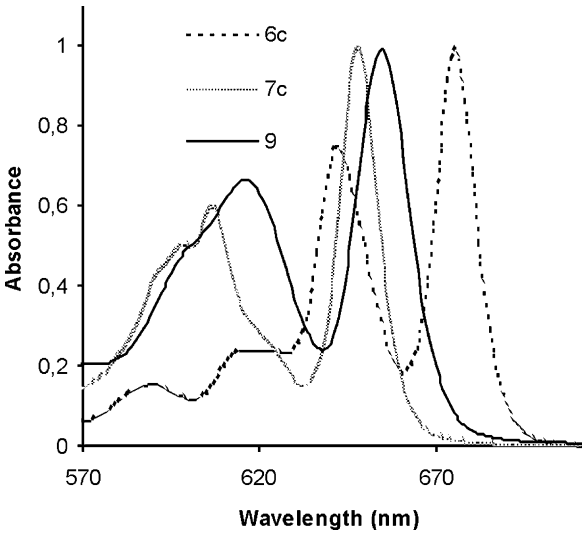


Fig. 4. Absorption spectra of **6c**, **7c** and **9** in pyridine.

compounds **6c** and **7c** have λ_{max} in the Q band region 673 and 648 nm, respectively. The compound **9** is blue shifted (with respect to **6c**) to 655 nm, which also corresponds to the ratio of substituents approximately 3:1.

The replacements on periphery made us search alternative ways of preparation. However, neither reaction with DBU nor with urea and zinc acetate in quinoline led to AzaPc's. We also tried the conversion of **3** to its isoindolinediimines with gas ammonia in absolute methanol, in the presence of catalytic amount of sodium methoxide. However, the yields after cyclization in formamide or dimethylaminoethanol of such prepared isoindolinediimine were very poor, 6 and 2% respectively. Also UV–Vis spectrum (not shown) was not satisfactory enough (λ_{max} 670 nm), perhaps due to small changes on periphery caused by catalytic amount of sodium methoxide used for preparation of isoindolinediimine. Finally, we tried cyclization with magnesium butanolate and it brought the desired product. Magnesium butanolate was found not to be strong enough to cause any changes in peripheral substitution. The compound was confirmed by CHN analysis and by comparing with UV–Vis spectrum of **10** (Fig. 5) (prepared by the same method from **3a**) that was confirmed by NMR spectroscopy (no signals of O–CH_2 were found in the ^{1}H NMR spectrum) (**6b** does not reach sufficient concentration in solution for NMR measurements). Compounds **10** and **6b** had the same UV–Vis spectrum since they differ only in the length of the side chain.

With respect to all the above-mentioned results we decided to choose as preparation method for metal-free AyaPc's **6c** and **7c** cyclization with magnesium butanolate, optimal reaction time 3 h, followed by removing Mg with strong acid. The procedure with lithium butanolate and 3 h of reaction time were chosen as conditions for preparation of **5c**.

Compounds **7** and **10** are well soluble in common organic solvents, e.g. chloroform, dichloromethane, toluene and pyridine. Solubility of **6** and **5** is much lower and does

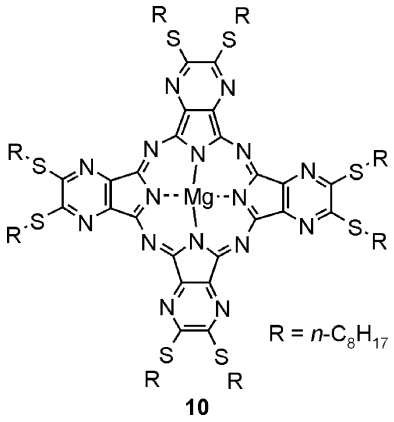


Fig. 5. Compound **10**.

not reach concentrations sufficient for NMR measurements but sufficient for singlet oxygen production tests. Properties of **9** are much closer to **7c** than **6c** as it is clear from ratio of substituents and therefore it is of increased solubility. Random arrangement of the different substituents on the macrocycle decreases symmetry of the compound **9** and it contributes to improved solubility of the AzaPc too.

Reaction times for conversion of magnesium AzaPc's to metal-free AzaPc's and this subsequently to zinc AzaPc's were determined also by observing changes in UV–Vis spectra of reaction mixture. Metal-free **5c**, **6c** and **7c** are split in Q band area into two bands (can be seen also on the Fig. 4), while metal AzaPc's do not and it simplifies the determination of the end of the reaction.

AzaPc's are known to coordinate molecules of solvents [11,14,15]; in our case, two molecules of water were also found in ^{1}H NMR spectra of compounds **7**, **9** and **10**. Depending on H-bonding with solvents used for measurement, its chemical shift (for **7b**) is δ 3.50 ppm (chloroform) or δ 2.48 ppm (tetrahydrofurane). UV–Vis spectra show typical B band (in region around 360 nm) and Q band (in region around 630–670 nm) and are shifted depending on the connecting heteroatom (e.g. 626 nm (**7b**), 651 nm (**5b**), 656 nm (**6b**, **10**)). Extinction coefficients are very high, especially for **6b** (287,100) and **10** (280,000).

2.3. Photodynamic properties

The ability to produce singlet oxygen (the main species in photodynamic action) was measured as a dye-sensitised photoxidation of 1,3-diphenylisobenzofurane-specific scavenger of singlet oxygen. Light under 550 nm was filtered off using filter, therefore the decomposition of DPBF in absence of the dye was minimal (maximum 3% after 10 min of irradiation). Results can be seen in Figs. 6 and 7. The photodynamic activity increase in order **5c**<**5b**<**5d**⋘<**7c**<**6c**≪**7b**<**6b**=**10**<**7d**<**6d**. As it has already been confirmed before [6], the best metal for PDT is zinc and metal-free AzaPc's are of poor activity. It is valid for all three types of substitution.

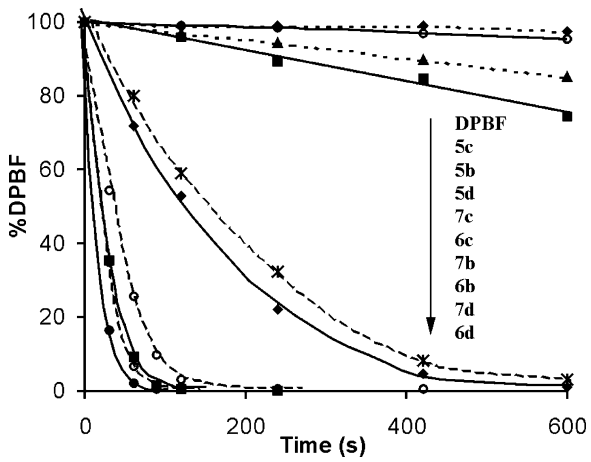


Fig. 6. DPBF degradation by singlet oxygen produced by dyes. Concentration of the dyes and DPBF was 5.0×10^{-6} and 50.0×10^{-6} mol/dm^3, respectively.

A very surprising finding is that compounds **5** are very poor producers of the singlet oxygen, when compared to **6** or **7**. The activity of zinc AzaPc's **5d**, the most potent amino derivative, is still far behind the activity of the least potent metal-free **6c** and **7c**. Compounds **6** and **7** possess comparable potency with a little higher activity for butylsulfanyl substances (Fig. 7). Compounds **6b** and **10** have completely the same UV–Vis spectral properties (absorption spectrum and even extinction coefficients) and the photodynamic activity is also the same (Fig. 7) as they differ only in the chain length. This matching shows that our starting hypothesis that four-carbon chain will be long enough to inhibit aggregation was right (aggregation lowers the extinction coefficients and inhibit efficient photodynamic action). On the other hand, this is valid only in low concentrations of **6b** because its solubility is evidently much lower than the solubility of **10**.

There were found neither qualitative nor quantitative changes in the absorption spectra of all dyes during the whole experiment, thus suggesting that neither decomposition nor aggregation took place. UV–Vis spectra of all dyes were sharp and the presence of no aggregates and dimers was found at concentrations chosen for measurements, even in maximal used dye concentration 5.0×10^{-6} mol/dm^3.

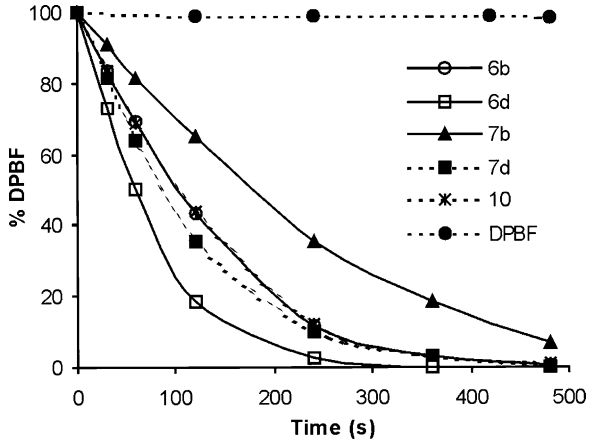


Fig. 7. DPBF degradation by singlet oxygen produced by the most potent dyes at 10 times lower concentration. Concentration of the dyes and DPBF was 0.5×10^{-6} and 50.0×10^{-6} mol/dm^3, respectively.

3. Conclusion

An optimal method and reaction time for preparation of AzaPc's **5–7** was found. The stability of peripheral chains against alkoholates during cyclization decreases from alkylamino (stable in both Li and Mg butanolates) through alkylsulfanyl (stable only in Mg butanolate) to alkoxy derivatives (unstable even in Mg butanolate [15]).

We analysed their potential for singlet oxygen production and discovered that compounds peripherally substituted with alkylamino substituents (**5**) were very poor in this property and were not suitable for PDT. That can be a reason for no activity in vitro of previously published compounds [6]. However, the reason for this behaviour remains still unclaimed and can be of interest for further research. On the other hand, alkylsulfanyl (**6**) and alkoxy (**7**) substituents are suitable for PDT since photodynamic activity of such AzaPc's is very high. From these two substituents alkylsulfanyl are promising for next research, because alkoxy substituents are not stable in alcoholates and it could cause problems during synthesis. Magnesium alkylsulfanyl AzaPc's are readily accessible in good yields and easily convertible to metal-free AzaPc's, a starting material for other metal AzaPc's. Other important advantages are the best photodynamic properties and the longest red shift of the Q band among all derivatives studied. These compounds, however are not soluble in water but their photodynamic activity can be measured after incorporation of the dyes into liposomes. Research on this "biological" part is in progress.

4. Experimental

All organic solvents used for the synthesis were of analytical grade. Butanol and dioxan were stored over sodium and distilled before use. TLC was performed on Silufol UV 254 plates (Kavalier, Votice). Merck Kieselgel 60 (0.040–0.063 mm) was used for column chromatography. Melting points were measured on Electrothermal IA9000 Series Digital Melting point Apparatus (Electrothermal Engineering Ltd., Southend-on-Sea, Essex, UK) and are uncorrected. The elementary analysis was carried out on Automatic Microanalyser EA1110CE (Fisons Instruments S.p.A., Milan, 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 and 75.43 MHz–^{13}C) Bruker Comp. (Karlsruhe, Germany). Chemical shifts reported are given relative to internal $Si(CH_3)_4$. NMR signals of **5–10** showed broadening. UV–Vis spectra were recorded on

spectrophotometer UV-2401PC, Shimadzu Europa GmbH (Duisburg, Germany).

4.1. Synthesis

4.1.1. 5,6-Dichloro-pyrazine-2,3-dicarbonitrile (1)

This compound was prepared according to previously published method [11].

4.1.2. 5,6-Bis-butylamino-pyrazine-2,3-dicarbonitrile (2)

A tetrahydrofurane (50 ml) solution of 1.00 g (5 mmol) of **1** was stirred at room temperature while 1.53 g (21 mmol) of butylamine was added dropwise. The solution was stirred and monitored on TLC (chloroform) until the reaction was completed. It took approximately 3 h. Precipitated hydrochloride of butylamine was filtered off and the rest was chromatographed on silica in chloroform and recrystalized from ethanol/water to yield 1.20 g (87%) of yellowish needles, mp 152 °C. IR (KBr) 2956, 2932, 2872, 2227 (C≡N), 1589, 1561, 1519. ^{13}C NMR ($CDCl_3$) δ 144.3, 119.7, 116.5, 41.9, 30.8, 20.4, 13.9. ^{1}H NMR ($CDCl_3$) δ 5.77–5.65 (m, 2H, NH), 3.56–3.44 (m, 4H, NH–CH_2), 1.74–1.62 (m, 4H, CH_2), 1.51–1.36 (m, 4H, CH_2), 0.95 (t, 6H, J = 7.0 Hz, CH_3). Anal. found C 61.48, H 7.78, N 30.53, Calc. ($C_{14}H_{20}N_6$) C 61.74, H 7.40, N 30.86.

4.1.3. 5,6-Bis-butylsulfanyl-pyrazine-2,3-dicarbonitrile (3)

A solution of 929 mg (10.3 mmol) of butanethiol in 10.2 ml of aq. NaOH (1.0 mol/dm^3) was stirred at room temperature for 30 min. Then, a solution of 1.00 g (5 mmol) of 1 in 20 ml of tetrahydrofurane was added at once. The reaction was stirred for another 30 min, evaporated, dissolved in dichloromethane and washed several times with water. Organic phase was then chromatographed on silica in toluene and recrystalized from methanol to yield 1.10 g (75%); of yellow needles, mp 56 °C. IR (KBr) 2961, 2933, 2872, 2232 (C≡N), 1478, 1287, 1150, 988. ^{13}C NMR ($CDCl_3$) δ 160.5, 126.0, 113.8, 31.0, 30.2, 21.9, 13.5. ^{1}H NMR ($CDCl_3$) δ 3.26 (t, 4H, J = 7.4 Hz, S–CH_2), 1.79–1.65 (m, 4H, CH_2), 1.56–1.41 (m, 4H, CH_2), 0.96 (t, 6H, J = 7.0 Hz, CH_3). Anal. found C 55.03, H 6.12, N 18.02, S 20.61 Calc. ($C_{14}H_{18}N_4S_2$) C 54.87, H 5.92, N 18.28., S 20.93.

4.1.4. 5,6-Bis-octylsulfanyl-pyrazine-2,3-dicarbonitrile (3a)

Was prepared from **1** with octanethiol similar to procedure mentioned by **3**. Yield 1.50 g (71%) of white needles, mp 64 °C. ^{13}C NMR ($CDCl_3$) δ 160.5, 126.1, 113.8, 31.8, 31.4, 29.1, 28.9, 28.8, 28.2, 22.6, 14.1. ^{1}H NMR ($CDCl_3$) δ 3.25 (t, 4H, J = 7.4 Hz, S–CH_2), 1.79–1.66 (m, 4H, CH_2), 1.51–1.39 (m, 4H, CH_2), 1.38–1.22 (m, 16H, CH_2), 0.94–0.83 (m, 6H, CH_3).

4.1.5. 5,6-Dibutoxy-pyrazine-2,3-dicarbonitrile (4)

A solution of 2.23 g (22 mmol) of triethylamine in 10 ml of dry butanol was stirred at room temperature for 30 min and then poured to a suspension of 2.00 g (10 mmol) of **1** in 20 ml of dry butanol. The suspension dissolved in a few minutes. Stirring was continued for 1 h at room temperature and then 2.5 h at reflux. The reaction was monitored on TLC (R_f = 0.46 in toluene). The solvent was removed under reduced pressure, the rest dissolved in toluene and washed several times with water. The organic phase was then chromatographed on silica with toluene to yield 1.35 mg (49%) of yellow crystals, mp 33–34 °C. IR (KBr) 2962, 2937, 2875, 2236 (C≡N), 1551, 1499, 1459, 1349, 1245. ^{13}C NMR ($CDCl_3$) δ 151.9, 122.5, 113.5, 69.2, 30.1, 18.9, 13.6. ^{1}H NMR ($CDCl_3$) δ 4.45 (t, 4H, J = 6.9 Hz, O–CH_2), 1.88–1.75 (m, 4H, CH_2), 1.54–1.39 (m, 4H, CH_2), 1.02–0.94 (m, 6H, CH_3). Anal. found C 61.58, H 6.79, N 20.13, Calc. ($C_{14}H_{16}N_4O_2$) C 61.30, H 6.61, N 20.42.

4.1.6. General procedure for synthesis of zinc azaphthalocyanines 5d, 6d and 7d

A metal-free azaphthalocyanine (0.1 mmol) was dissolved in hot dimethylformamide (**5c**) or hot dimethylformamide/toluene (1:1) (**6c**, **7c**) and 219 mg (1 mmol) of zinc acetate hydrate were added. Heating was continued for 1 h. After this time, solvents were removed under reduced pressure and dark solid washed thoroughly with hot water to yield a crude azaphthalocyanines **5d**, **6d**, **7d** that were purified by methods given below.

4.1.7. {29H,31H-[2,3,9,10,16,17,23,24-Octakis(butylamino)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato](2-)-N^{29},N^{30},N^{31},N^{32}}magnesium (II) (5b)

Thirty-five milligram (5.3 mmol) of magnesium with a small crystal of iodine were refluxed in 2 ml of dry butanol for 3 h and then 204 mg (0.75 mmol) of **2** were added. Refluxing continued for next 24 h. After evaporation to dryness, 50 ml of 50% aq. acetic acid were poured into and thus the obtained suspension was stirred for 0.5 h. Dark blue solid was filtered off and washed with hot water, acetone, chloroform and several portions of methanol. The product was then dissolved in minimal amount of acetic acid (98%) and poured on ice. Fine blue precipitate was centrifuged and washed with water and acetone. Thus, gained blue solid was of satisfactory purity. Yield 53 mg (26%), mp >300 °C. IR (KBr) 2958, 2932, 2871, 1597, 1534, 1480, 1466, 1369. Anal. found C 58.87, H 7.62, N 28.96, Calc. ($C_{56}H_{80}MgN_{24} + 2H_2O$) C 58.50, H 7.36, N 29.24. UV–Vis (pyridine): λ_{max} (ε) 651 (172600), 595 (27400), 510 (37100), 373 nm (134400).

4.1.8. 29H,31H-[2,3,9,10,16,17,23,24-Octakis(butylamino)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato](2-)-N^{29},N^{30},N^{31},N^{32}}2H (5c)

Dry butanol (10 ml) with 952 mg (3.5 mmol) of **3** was heated to reflux and then 168 mg (24 mmol) of metal lithium were inserted. Mixture was heated for another 3 h. After this time, the solution was evaporated to dryness and purified similar to **5b**. Yield 230 mg (24%) of purple solid, mp >300 °C. IR (KBr) 2958, 2932, 2871, 1597, 1528, 1484,

1467, 1368. Anal. found C 59.94, H 7.92, N 29.58, Calc. ($C_{56}H_{82}N_{24} + 2H_2O$) C 59.66, H 7.69, N 29.82. UV–Vis (pyridine): λ_{max} (ε) 675 (66600), 640 (46500), 512 (63800), 361 nm (117000).

*4.1.9. {29H,31H-[2,3,9,10,16,17,23,24-Octakis(butylamino)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato](2-)-N^{29},N^{30},N^{31},N^{32}}zinc (II) (**5d**)*

Crude **5d** was purified according to the procedure mentioned at **5b** (but without acetic acid in the beginning). Yield 87 mg (76%) of blue solid, mp >300 °C. IR (KBr) 2958, 2932, 2871, 1596, 1530, 1483, 1466, 1367. Anal. found C 56.68, H 7.33, N 28.02, Calc. ($C_{56}H_{80}ZnN_{24} + 2H_2O$) C 56.48, H 7.11, N 28.23. UV–Vis (pyridine): λ_{max} (ε) 651 (82300), 597 (17700), 522 (23200), 377 nm (109000).

*4.1.10. {29H,31H-[2,3,9,10,16,17,23,24-Octakis(butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato](2-)-N^{29},N^{30},N^{31},N^{32}}magnesium (II) (**6b**)*

Five hundred and ten miligram (21 mmol) of magnesium and a small crystal of iodine were refluxed for 3 h in dry butanol and then 918 mg (3 mmol) of **3** were added. The mixture was refluxed for another 3 h and evaporated to dryness. Fifty millilitre of 50% aq. acetic acid were added and the suspension was stirred for 0.5 h. The green solid was then filtered off, thoroughly washed with water, methanol and hot acetone and finally recrystallized twice from hot pyridine. Yield 600 mg (64%) of green solid, mp >300 °C. IR (KBr) 2958, 2930, 2871, 1637, 1509, 1464, 1258, 1232, 1164, 1099, 977. Anal. found C 52.52, H 5.85, N 17.55, S 19.81, Calc. ($C_{56}H_{72}MgN_{16}S_8 + 2H_2O$) C 52.30, H 5.96, N 17.42, S 19.94. UV–Vis (pyridine): λ_{max} (ε) 656 (287100), 594 (37400), 386 nm (141600).

*4.1.11. {29H,31H-[2,3,9,10,16,17,23,24-Octakis(butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato](2-)-N^{29},N^{30},N^{31},N^{32}}2H (**6c**)*

Magnesium AzaPc **6b** (490 mg, 0.4 mmol) was dissolved in 80 ml of hot chloroform and a solution of 1.20 g (6 mmol) of *p*-toluenesulphonic acid in 10 ml of tetrahydrofurane was added. A resulting mixture was stirred at r.t. for 1.5 h, evaporated and thoroughly washed with hot water, methanol and acetone. The product was finally recrystalized twice from hot pyridine. Yield 448 mg (93%) of green solid (before recrystalization), 310 mg (64%) (after recrystalization), mp >300 °C. IR (KBr) 2958, 2930, 2872, 1637, 1515, 1464, 1427, 1162. Anal. found C 53.45, H 5.96, N 17.42, S 20.02 Calc. ($C_{56}H_{74}N_{16}S_8 + 2H_2O$) C 53.22, H 6.22, N 17.73, S 20.30. UV–Vis (pyridine): λ_{max} (ε) 673 (124500), 648 (113800), 592 (24600), 480 (42100), 375 nm (107700).

*4.1.12. {29H,31H-[2,3,9,10,16,17,23,24-Octakis(butylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato](2-)-N^{29},N^{30},N^{31},N^{32}}zinc (II) (**6d**)*

Crude **6d** was purified according to the procedure mentioned at **6b** (but without acetic acid). Yield 119 mg (88%) of dark blue solid, mp >300 °C. IR (KBr) 2958, 2930, 2871, 1637, 1512, 1453, 1257, 1233, 1162. Anal. found C 51.05, H 5.58, N 16.64, S 18.99, Calc. ($C_{56}H_{72}ZnN_{16}S_8 + 2H_2O$) C 50.68, H 5.77, N 16.89, S 19.33. UV–Vis (pyridine): λ_{max} (ε) 655 (251100), 593 (32400), 460 (24900), 385 nm (134200).

*4.1.13. {29H,31H-[2,3,9,10,16,17,23,24-Octakis(butoxy)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato](2-)-N^{29},N^{30},N^{31},N^{32}}magnesium (II) (**7b**)*

Five hundred and ten milligrams (21 mmol) of magnesium and a small crystal of iodine were refluxed for 3 h in dry butanol and then 822 mg (3 mmol) of **4** were added. The mixture was refluxed for another 3 h and evaporated to dryness. 50 ml of 50% aq. acetic acid were added and the suspension was stirred for 0.5 h. The green solid was then filtered off and thoroughly washed with water, methanol and hot acetone. A crude product was absorbed to silica and washed on a glass frit with methanol until the solution was colourless. The product on silica was then poured on silica column and chromatographed with chloroform, evaporated and finally washed with acetone to yield 490 mg (58%) of the dark blue solid, mp >300 °C. IR (KBr) 2959, 2934, 2873, 1639, 1542, 1446, 1378, 1306, 1254. ^{13}C NMR ($CDCl_3$) δ 151.9, 147.1, 138.7, 67.9, 30.6, 19.2, 13.9. ^{1}H NMR ($CDCl_3$) δ 5.08–3.55 (m, 16H, O–C*H*$_2$), 3.54–2.60 (bs, 4H, H_2O), 1.96–1.54 (m, 16H, CH_2), 1.52–1.12 (m, 16H, CH_2), 1.12–0.64 (m, 24H, CH_3). Anal. found C 58.50, H 6.82, N 18.96, Calc. ($C_{56}H_{72}MgN_{16}O_8 + 2H_2O$) C 58.10, H 6.62, N 19.36. UV–Vis (pyridine): λ_{max} (ε) 626 (208400), 569 (27200), 368 nm (115300).

*4.1.14. {29H,31H-[2,3,9,10,16,17,23,24-Octakis(butoxy)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato](2-)-N^{29},N^{30},N^{31},N^{32}}2H (**7c**)*

Compound **7c** was prepared similar to **6c** with following differences: 336 mg (0.3 mmol) of **7b** were dissolved in 10 ml of chloroform at r.t. and the product was not finally recrystalized from pyridine. Yield 224 mg (68%) of dark brown solid, mp >300 °C. IR (KBr) 2959, 2935, 2874, 1640, 1540, 1456, 1381, 1317, 1251. ^{13}C NMR ($CDCl_3$) δ 68.6, 31.0, 19.6, 14.2 (and broad multiplet aromatic signal about 153.5). ^{1}H NMR ($CDCl_3$) δ 5.52–3.84 (m, 16H, O–C*H*$_2$), 3.82–2.64 (bs, 4H, H_2O), 2.46–1.98 (m, 16H, CH_2), 1.98–1.54 (m, 16H, CH_2), 1.54–0.92 (m, 24H, CH_3). Anal. found C 59.58, H 7.14, N 13.73, Calc. ($C_{56}H_{74}N_{16}O_8 + 2H_2O$) C 59.24, H 6.92, N 14.09. UV–Vis (pyridine): λ_{max} (ε) 648 (97400), 606 (73200), 556 (19400), 425 (32800), 346 nm (93000).

*4.1.15. {29H,31H-[2,3,9,10,16,17,23,24-Octakis(butoxy)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato](2-)-N^{29},N^{30},N^{31},N^{32}}zinc (II) (**7d**)*

Crude **7d** was purified according to procedure mentioned by **7b** (but without acetic acid) to yield 80 mg (70%) of dark blue solid, mp >300 °C. IR (KBr) 2960, 2935, 2874, 1638, 1542, 1446, 1378, 1305, 1255. ^{13}C NMR ($CDCl_3$)

δ 152.1, 148.7, 139.7, 67.9, 30.8, 19.4, 14.0. ^{1}H NMR ($CDCl_3$) δ 5.32–3.85 (m, 16H, O–CH_2), 3.80–2.85 (bs, 4H, H_2O), 2.37–1.75 (m, 16H, CH_2), 1.75–1.31 (m, 16H, CH_2), 1.31–0.50 (m, 24H, CH_3). ^{1}H NMR (tetrahydrofurane) δ 4.44–3.49 (m, 16H, O–CH_2), 2.84–2.17 (bs, 4H, H_2O), 1.91–1.41 (m, 16H, CH_2), 1.41–1.08 (m, 16H, CH_2), 1.08–0.46 (m, 24H, CH_3). Anal. found C 56.48, H 6.59, N 18.33, Calc. ($C_{56}H_{72}ZnN_{16}O_8$+2H_2O) C 56.11, H 6.39, N 18.70. UV–Vis (pyridine): λ_{max} (ε) 624 (202900), 567 (26900), 368 nm (128400).

4.1.16. Compound **9**

One hundred and seven milligram (0.35 mmol) of **3** were heated in dry butanol (3 ml) to reflux and 17 mg (2.4 mmol) of metal lithium were inserted. The mixture was kept refluxing for next 3 h and evaporated to dryness. The product was then purified as described for **7b**. Yield 35 mg of green solid, mp >300 °C. ^{1}H NMR ($CDCl_3$) δ 5.24–4.84 (m, 12H, O–CH_2), 3.90–3.58 (m, 4H, S–CH_2), 3.49 (bs, 4H, H_2O), 2.35–2.01 (m, 16H, CH_2), 1.97–1.73 (m, 16H, CH_2), 1.33–1.18 (m, 24H, CH_3). UV–Vis (chloroform): λ_{max} 655, 616, 440, 352 nm.

4.1.17. {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}}magnesium (II) (**10**)

A product was prepared from 1.25 g (3 mmol) of **3a** and 510 mg (21 mmol) of magnesium and purified according to procedure mentioned by **7b**. Yield 800 mg (63%) of green solid, mp > 300 °C. ^{13}C NMR ($CDCl_3$) δ 157.7, 149.5, 143.8, 32.0, 31.4, 29.6, 29.4, 29.3, 28.4, 22.8, 14.2. ^{1}H NMR ($CDCl_3$) δ 3.60–2.66 (m, 16H, S–CH_2), 2.60 (bs, 4H, H_2O), 2.00–1.58 (m, 16H, CH_2), 1.58–1.04 (m, 80H, CH_2), 1.04–0.76 (m, 24H, CH_3). UV–Vis (pyridine): λ_{max} (ε) 656 (280000), 594 (36000), 385 nm (138000).

4.2. Measurements of reaction time for preparation of AzaPc's

4.2.1. Magnesium azaphthalocyanines (**5b**, **6b**, **7b**)

Dry butanol (2.5 ml), metal magnesium (42 mg, 1.75 mmol) and a small crystal of iodine were heated under reflux for 3 h. Then, 0.25 mmol of precursor (**2**, **3** or **4**) was added. Refluxing continued and samples (10 μl) were taken at given times (always twice for each measurement), dissolved in chloroform (**6b**, **7b**) or dimethylformamide (**5b**) to suitable concentration and UV–Vis spectra of such a prepared solutions of known concentration were measured. The yields were counted later using extinction coefficients of the AzaPc's at λ_{max} of their Q bands.

4.2.2. Metal-free azaphthalocyanines (**5c**, **7c**)

Dry butanol (2.5 ml) and 0.25 mmol of precursor (**2** or **4**) were heated to reflux and 12.5 mg (1.75 mmol) of metal lithium was inserted. The mixture was heated to reflux and samples (10 μl) were taken at given times (always twice for each measurement), poured into 0.2 ml of acetic acid, dissolved in chloroform (**7c**) or dimethylformamide (**5c**) to suitable concentration and UV–Vis spectra of such a prepared solution of known concentration were measured. The yields were counted later using extinction coefficients of the AzaPc's at λ_{max} of their Q bands.

4.3. Singlet oxygen measurements

Singlet oxygen measurements were carried out by a DPBF decomposition reaction. AzaPc's (5.0×10^{-6} and 0.5×10^{-6} mol/dm^3) and DPBF (50.0×10^{-6} mol/dm^3) were dissolved in pyridine, in the dark transferred to a glass tube and during vigorous stirring irradiated from a distance 0.5 m for different times. Light under 550 nm was filtered off using orange HOYA G filter. As a light source a halogen lamp (OSRAM, 500 W) was used. A decrease of DPBF concentration was followed by an absorbance at 417 nm.

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