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The synthesis and characterization of novel unsymmetrical azaphthalocyanines containing one carboxylic group

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Abstract

Unsymmetrical zinc and magnesium complexes of azaphthalocyanines with one carboxylic group were synthesized using statistical condensation of 5,6-bis(*tert*-butylsulfanyl)pyrazine-2,3-dicarbonitrile (A) and 3-(5,6-dicyano-3-methylpyrazin-2-ylsulfanyl)propionic acid (B) or 6-(3-*tert*-butylsulfanyl-5,6-dicyanopyrazine-2-ylamino)hexanoic acid (C); unsymmetrical AAAB or AAAC azaphthalocyanines were isolated from the mixture. During preparation of Mg complexes of AAAC type, the precursor C had to be esterified before cyclotetramerization and the final azaphthalocyanine was hydrolyzed to yield AAAC of higher purity. The synthesized compounds were characterized using IR, NMR, MS and UV–vis spectroscopy; singlet oxygen quantum yields were measured using DPBF decomposition method. The zinc AAAB complex showed good photodynamic properties indicating that it may be suitable as a third generation photosensitizer.

Keywords: Unsymmetrical substitution; Azaphthalocyanine; Tetrapyrazinoporphyrazine; Monofunctional; Singlet oxygen quantum yield; Carboxylic acid

1. Introduction

In recent years, phthalocyanines and their analogs have become a subject of increasing research interest [1], as commercial dyes, in photovoltaic applications [2] or for non-linear optics [3]. Despite their strong absorption in the far red region, which makes them suitable as potential photosensitizers in photodynamic therapy (PDT) [4–7], this particular application requires higher selectivity to tumor cells. In this context, one approach involves the combination of a photosensitizer with a targeting moiety; compounds of this type are referred to as third generation photosensitizers. The targeting component improves localization of the dye in specific sites and thus increases selectivity, tumor uptake and, consequently, PDT effectiveness; several types of targeting compounds are under investigation and the uses of folic acid [8], polylysine chains [9,10], monoclonal antibodies [11] and steroids [12] have been reported.

Although the syntheses of derivatizable symmetrical phthalocyanines and their aza-analogs from one precursor is relatively straightforward, it leads to compounds containing four (in the case of monosubstituted precursor) or eight (in the case of disubstituted precursor) derivatizable groups (e.g. carboxy- or amino groups). The presence of more than one modifiable group could lead to polymerization; in addition, complex mixtures can arise during conjugation with the targeting compound. Whilst the presence of one functionality seems therefore to be more suitable [13,14], this involves the more complicated syntheses of unsymmetrical compounds that require specific approaches [15,16]—a subphthalocyanine method [17,18], polymeric support method [19–21] or statistical condensation [13,14]. Whilst the latter method is most widely used the ensuing mixture of different Pcs must be chromatographically resolved, which is often problematic owing to the low solubility of Pc in many solvents and the tendency of these planar macrocyclic systems to aggregate. Peripheral

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substitution of the Pc macrocycle with bulky substituents prevents this behavior [22,23] allowing chromatographic separation of the desired compounds from admixture [4].

This work concerns the preparation of azaphthalocyanines (AzaPc) containing one carboxylic group. Such molecules can then be combined with targeting moieties to form third generation photosensitizers.

2. Experimental

All organic solvents used for the synthesis were of analytical grade. Anhydrous butanol was stored over magnesium and distilled prior to use. Anhydrous dimethylformamide (DMF) was purchased from Acros. 1,3-Diphenylisobenzofuran (DPBF), 2,2-dimethyl-propane-1-thiol and 3-sulphanylpropionic acid were purchased from Aldrich. 6-Aminohexanoic acid was purchased from Fluka. Zinc phthalocyanine (ZnPc) was obtained from Eastman Organic Chemicals (New York, USA). All chemicals were used as received except for zinc acetate dihydrate (Lachema, Czech Republic), which was dried at 78 °C under reduced pressure (13 mbar) for 5 h. TLC was performed on Merck aluminium sheets with silica gel 60 F_{254}. 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. 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). 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). The elemental analysis was carried out on an Automatic Microanalyser EA1110CE (Carlo Erba S.p.A., Milano, Italy). MALDI-TOF mass spectra were recorded in positive reflectron mode on a mass spectrometer Voyager-DE STR (Applied Biosystems, Framingham, MA, USA). For each sample, 0.5 μL of the mixture was spotted onto the target plate, air-dried and covered with 0.5 μL of matrix solution consisting of 10 mg of α-cyano-4-hydroxycinnamic acid in 100 μL of 50% ACN in 0.1% trifluoroacetic acid. The instrument was calibrated externally with a five-point calibration using Peptide Calibration Mix1 (LaserBio Labs, Sophia-Antipolis, France). ESI MS spectra of compound **6** were measured on Quattro Micro™ API (Waters, Milford, MA, USA) in positive electrospray mode. Solution for ESI MS was prepared in methanol and formic acid was added before measurements to support ionization.

Compounds **1** [22], **2** [24], **4** and **5** [25] were prepared according to published procedures in good purity. Compounds **10** and **11** that were isolated from the reaction mixtures showed the same characteristics (R_f values, UV–vis, NMR, IR spectra) as the same compounds prepared before by simple tetramerization of **1** [22], and therefore they are not characterized here.

2.1. 3-(5,6-Dicyano-3-methylpyrazin-2-ylsulfanyl) propionic acid (*3*)

A 1.0 M aqueous solution of NaOH (2.2 mL) was stirred at r.t. and 3-sulphanylpropionic acid (117 mg, 1.1 mmol) was added. The mixture was stirred for 15 min and a solution of **2** (178 mg, 1 mmol) in acetone (10 mL) was added dropwise. The reaction mixture was stirred at r.t. for 1 h with monitoring on TLC using chloroform as a mobile phase. After the reaction was completed, the organic part was evaporated under reduced pressure, and the aqueous solution of **3** was acidified with a few drops of concentrated HCl. The precipitated solid was collected and washed with water. The crude product was purified on silica gel using chloroform/acetic acid 10:1 as the eluent. Yield 98 mg (39%) of light yellow solid; m.p. 143–144 °C; IR, ν (cm^{-1}): 2956, 2862 (alkyl CH), 2234 (C≡N), 1701 (C=O); ^{1}H NMR (acetone-d_6) δ: 2.59 (s, 3H, CH_3), 2.82 (t, 2H, $J = 6.4$ Hz, CH_2), 3.52 (t, 2H, $J = 6.7$ Hz, S–CH_2); ^{13}C NMR (acetone-d_6) δ: 22.1, 26.2, 33.1, 114.6, 114.9, 127.5, 131.0, 157.3, 162.9, 172.7. Anal. calcd for $C_{10}H_8N_4O_2S$: C 48.38; H 3.25; N 22.57; S 12.92%. Found: C 48.05; H 3.28; N 22.42; S 12.59.

2.2. 3,9,10,16,17,23,24-Heptakis(tert-butylsulfanyl)-2-(5-carboxypentylamino)-1,4,8,11,15,18,22,25-octaazaphthalocyaninato magnesium(II) (*6*)

Magnesium (343 mg, 14 mmol) and a small crystal of iodine were refluxed in anhydrous butanol (20 mL) for 4 h. Compounds **1** (459 mg, 1.5 mmol) and **5** (202 mg, 0.5 mmol) were added and the reflux continued for next 2 h. After this time, the dark green solution was cooled down and evaporated. The solid was then suspended in 50% (v/v) acetic acid and stirred at r.t. for 30 min. The green solid was collected and washed thoroughly with water. This mixture of AzaPc was not separated but hydrolyzed immediately. Thus, the green solid was dissolved in THF (50 mL) and a 0.1 M solution (90 mL) of KOH (9 mmol) in water/ethanol (1:10) was added. The reaction was stopped after 24 h of stirring at r.t. and the solvents were evaporated. The solid was suspended in 20% (v/v) acetic acid, stirred for 30 min and collected by filtration with a subsequent washing with water. The product was isolated using column chromatography on silica with chloroform/acetone/methanol 30:1:1 as the eluent. The first intense green fraction corresponds to compound **10**. The next intense green fraction corresponding to product **6** was collected and purified on silica once more with the same eluent. Yield 46 mg (7%); IR, ν (cm^{-1}): 2959, 2922, 2858 (alkyl CH), 1709 (C=O), 1567, 1516, 1456, 1362, 1252, 1233, 1144; ^{1}H NMR (pyridine-d_5) δ: 7.36 (t, 1H, $J = 5.4$ Hz, NH), 4.36 (q, 2H, $J = 6.5$ Hz, NH–CH_2), 2.70–2.57 (m, 2H, CH_2COO), 2.30–2.18 (m, 54H, CH_3), 2.15 (s, 9H, CH_3), 2.09–1.97 (m, 4H, CH_2), 1.97–1.84 (m, 2H, CH_2); ^{13}C NMR (pyridine-d_5) δ: 175.92, 158.74, 158.29, 158.09, 157.99, 157.56, 153.64, 153.25, 152.79, 151.43, 150.74, 150.67, 148.25, 147.06, 145.75, 145.73, 145.59, 145.40, 145.38, 145.27, 140.45, 51.33, 51.29, 51.16, 50.86, 42.58,

35.09, 30.95, 30.91, 30.80, 29.98, 27.63, 25.57; MS (ESI) *m/z* 1323 [M + H$^+$ + MeOH], 1313 [M + Na$^+$], 1291 [M + H$^+$], 1235, 1179, 1123, 1067, 1011, 955, 899. Anal. calcd for $C_{58}H_{75}MgN_{17}O_2S_7 \cdot 3H_2O$: C 51.79; H 6.07; N 17.70; S 16.69%. Found: C 51.68; H 6.11; N 17.76; S 16.51. UV–vis λ_{max} (nm) (ε) in DMF: 659 (151,400), 598 (30,400), 377 (132,000); UV–vis λ_{max} (nm) (ε) in tetrahydrofuran: 655 (165,400), 593 (30,000), 382 (130,200).

*2.3. 3,9,10,16,17,23,24-Heptakis(tert-butylsulfanyl)-2-(5-carboxypentylamino)-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc(II) (**8**)*

A mixture of **1** (612 mg, 2 mmol), **4** (233 mg, 0.66 mmol) and anhydrous zinc acetate (1.83 g, 10 mmol) was stirred in anhydrous DMF (10 mL) at 160 °C for 2.5 h. The reaction mixture was cooled down and poured into water (150 mL). A fine dark green precipitate was collected by filtration and washed thoroughly with water. The product was isolated using column chromatography on silica with chloroform/acetone/methanol 60:2:1 as the eluent. The first intense green fraction corresponds to compound **11**. After the compound **11** was eluted from column, the mobile phase was changed to chloroform/acetone/methanol 20:1:1 to obtain fractions containing compound **8**. The fractions corresponding to product were purified on silica once more with the former eluent (60:2:1). Yield 42 mg (5%); IR, ν (cm^{-1}): 2958, 2922, 2858 (alkyl CH), 1708 (C=O), 1567, 1517, 1456, 1362, 1253, 1232, 1144; ^{1}H NMR (pyridine-d_5) δ: 7.92 (t, 1H, $J = 5.8$ Hz, NH), 4.57–4.40 (m, 2H, NH–CH_2), 2.78 (t, 2H, $J = 6.4$ Hz, CH_2–COO), 2.35 (s, 54H, CH_3), 2.27 (s, 9H, CH_3), 2.24–2.10 (m, 4H, CH_2), 2.09–1.95 (m, 2H, CH_2); ^{13}C NMR (pyridine-d_5) δ: 175.88, 158.95, 158.53, 158.34, 158.26, 157.86, 153.70, 153.23, 151.97, 151.24, 150.90, 147.70, 147.52, 145.14, 145.09, 144.95, 144.77, 144.72, 147.62, 139.90, 51.39, 51.35, 51.25, 51.23, 50.91, 42.59, 35.06, 30.92, 30.88, 30.77, 29.96, 27.64, 25.58; MS (MALDI-TOF) *m/z* 1330 [M + H$^+$]. Anal. calcd for $C_{58}H_{75}N_{17}O_2S_7Zn \cdot 3H_2O$: C 50.26; H 5.89; N 17.18; S 16.19%. Found: C 50.18; H 5.94; N 17.03; S 16.01. UV–vis λ_{max} (nm) (ε) in DMF: 658 (135,600), 598 (25,700), 384 (105,700).

*2.4. 9,10,16,17,23,24-Hexakis(tert-butylsulfanyl)-2-(2-carboxyethylsulfanyl)-3-methyl-1,4,8,11,15,18,22,25-octaazaphthalocyaninato zinc(II) (**9**)*

A mixture of **1** (612 mg, 2 mmol), **3** (164 mg, 0.66 mmol) and anhydrous zinc acetate (1.83 g, 10 mmol) was stirred in anhydrous DMF (10 mL) at 160 °C for 2.5 h. The reaction mixture was cooled down and poured into water (150 mL). A fine dark green precipitate was collected by filtration and washed thoroughly with water. The product was isolated using column chromatography on silica with chloroform/acetone/methanol 15:1:1 as the eluent. The first intense green fraction corresponds to compound **11**. The next intense green fraction corresponding to product **9** was collected and purified on silica once more with chloroform/tetrahydrofuran 2:1. Yield 10 mg (2%); IR, ν (cm^{-1}): 2961, 2919, 2861 (alkyl CH), 1711 (C=O), 1522, 1455, 1363, 1258, 1233, 1145; ^{1}H NMR (pyridine-d_5) δ: 4.42 (t, 2H, $J = 6.8$ Hz, S–CH_2), 3.77 (t, 2H, $J = 6.7$ Hz, CH_2–COO), 2.99 (s, 3H, CH_3), 2.40–2.11 (m, 54H, $SCCH_3$); ^{13}C NMR (pyridine-d_5) δ: 174.76, 159.54, 158.94, 158.89, 158.74, 158.71, 158.57, 158.15, 153.18, 151.86, 151.83, 151.76, 151.63, 151.43, 150.99, 149.00, 148.68, 145.95, 145.40, 145.23, 145.06, 145.02, 144.98, 51.44, 51.38, 51.30, 51.06, 35.09, 30.83, 30.75, 30.66, 26.64, 22.65; MS (MALDI-TOF) *m/z* 1253 [M + Na$^+$], 1231 [M + H$^+$], 1189, 1175, 1119, 1063. Anal. calcd for $C_{52}H_{62}N_{16}O_2S_7Zn \cdot 3H_2O$: C 48.53; H 5.33; N 17.41; S 17.44%. Found: C 48.26; H 5.45; N 17.10; S 17.38. UV–vis λ_{max} (nm) (ε) in DMF: 654 (228,300), 594 (31,000), 387 (127,200).

2.5. Singlet oxygen measurements

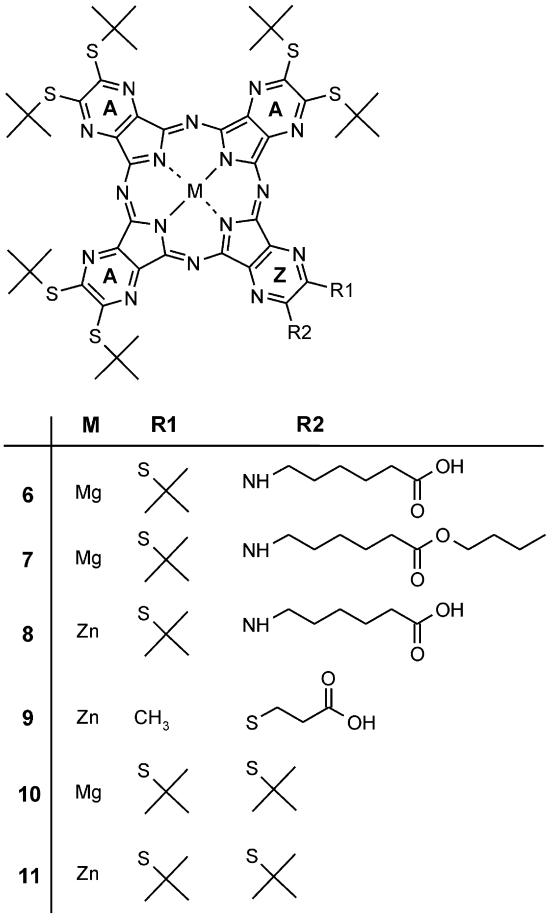
Singlet oxygen production was monitored as DPBF decomposition reactions, as reported by us previously [25]. Shortly, 2.5 mL of a stock solution of DPBF in DMF (5×10^{-5} M) was transferred into a 10 × 10 mm quartz optical cell and bubbled with oxygen for 1 min. DMF stock solution of the tested dye (usually 30 μL) was then added. Absorbance of the dye solution in Q-band maximum was always about 0.1. The solution was then stirred and irradiated for defined times using a halogen lamp (Tip, 200 W). Incident light was filtered through a water filter (6 cm) and an orange HOYA G filter to remove infrared light and light under 506 nm, respectively. A decrease of DPBF amount in the solution (maximum 15%) was measured as the decrease in its absorbance at 415 nm. Calculations of singlet oxygen quantum yield were then performed according to published equations [25]. ZnPc in DMF was used as the reference ($\Phi_\Delta = 0.56$ [4,26]).

3. Results and discussion

3.1. Synthesis

We have designed the final structures in accordance with our former investigations on structure–activity relationships in the group of AzaPc [22,25,27]. Bulky *tert*-butylsulfanyl substituents prevent efficiently the aggregation and, at the same time, they have positive effect on the production of singlet oxygen. That is why they were chosen to form three quarters (A) of the final AzaPc (Scheme 1) macrocycle. The fourth quarter (Z) is formed by substituents with one free carboxylic acid. Compounds **1** and **3** or **4** (Scheme 2) were chosen as precursors for the cyclotetramerization. During preparation of AzaPc **6**, a starting precursor **4** had to be replaced later by **5** because of the synthetic reasons (see below). Compounds **1**, **4** and **5** have already been prepared in our laboratory before. Compound **3** was synthesized using a simple nucleophilic substitution of chlorine atom in **2** with an appropriate thiolate.

The final AzaPc (Scheme 1) were prepared by means of statistical condensation with subsequent chromatographic isolation of the desired product. Isolation of the desired AzaPc **6**,



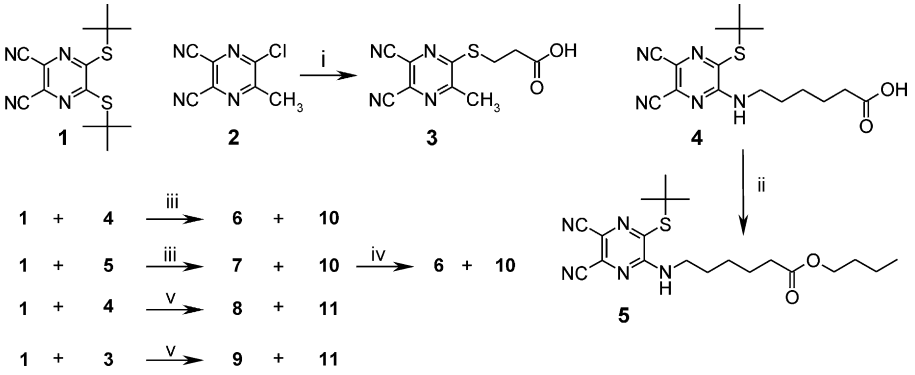
Scheme 1. Structures of prepared AzaPc.

8 and **9** using silica gel columns was not very difficult. Their R_f values were sufficiently different from lipophilic compounds (**10** or **11**) or the congeners containing two or more carboxylic groups.

One of the standard procedures for AzaPc macrocycle formation involves reaction in butanol in the presence of butoxides [28,29]. This cyclization method usually proceeds in high yields. However, some of the labile peripheral substituents can be exchanged for butoxy ones during cyclotetramerization due to high nucleophilicity of butoxide (or generally alkoxide) anion [27,30,31]. Alkylsulfanyl substituents were found to be labile in the presence of lithium butoxide but stable against magnesium butoxide attack [27]. That is why we have chosen the magnesium butoxide as the cyclization agent. Surprisingly, a strong typical odor of 2,2-dimethylpropanethiol appeared after statistical condensation of **1** and **4**. This indicates that some of the peripheral *tert*-butylsulfanyl substituents may be replaced by the butoxy ones. A TLC analysis of this statistical mixture showed two main products corresponding to symmetrical **10** and desired compound with one carboxylic group (**6**). However, a careful TLC analysis disclosed that the TLC spot of **10** consisted of three or four overlapping green compounds. Similar replacement is likely to happen also in compound **6** but TLC showed only one intense spot. The presence of side product was confirmed after the isolation of **6**. ESI MS analysis showed the presence of not only molecular ion peak of **6** at expected $m/z = 1291$ $[M + H^+]$ but also a minor peak of the compound with one butoxy group instead of *tert*-butylsulfanyl at $m/z = 1275$.

The changes described above were not expected because such behavior (odor or changes on TLC) was not observed during separate condensation of **1** to **10** [22]. Just to be sure, we prepared compound **10** in a separate reaction directly from **1** in magnesium butoxide again but no odor or changes on TLC were found. The fact that the changes occur only when **4** is in reaction leads us to think about the importance of the carboxylic moiety in the process. We have repeated the statistical condensation with a modified minor building block having the carboxyl group of **4** blocked by esterification (Scheme 2, reaction of **1** with **5**). No odor of 2,2-dimethylpropanethiol was observed. The R_f values of **10** and **7** (and other possible derivatives) are very similar, and the final statistical mixture cannot be directly separated. The ester bonds were therefore hydrolyzed using KOH, and compound **6** was isolated in a pure form.

Magnesium complexes of AzaPc can readily be converted to metal-free compounds, and subsequently various central metals can be inserted into the centre of macrocycle [22,29]. Due to the problems described above in the preparation of magnesium complexes, we decided to use rather a direct



Scheme 2. Reaction conditions: (i) 3-sulphanylpropionic acid, NaOH/water, acetone, r.t.; (ii) $SOCl_2$, THF, reflux, butanol, pyridine, reflux; (iii) Mg butoxide, butanol, iodine, reflux; (iv) KOH, ethanol/water, r.t.; (v) DMF, zinc acetate, reflux.

cyclotetramerization of precursors in DMF with zinc acetate resulting in the zinc complexes **8** and **9**. This cyclization runs with lower yields but no changes on the periphery occur.

3.2. Characterization

All prepared unsymmetrical AzaPc **6**, **8** and **9** showed excellent solubility in common laboratory solvents (acetone, chloroform, dichloromethane, pyridine, DMF, tetrahydrofuran, toluene) due to the presence of aggregation inhibiting substituents. The high lipophilicity caused by six or seven *tert*-butylsulfanyl substituents is partially decreased by the carboxyl group thus allowing better solubility also in more polar solvents like methanol, ethanol and acetonitrile. Such good solubility of the new AzaPc in a wide range of solvents makes them prospective candidates for conjugation reactions. High solubility will be advantageous and will allow the reactions to run in the solution and not in the suspension.

The IR spectra of precursors showed characteristic C≡N stretch vibrations around 2230 cm^{-1}. The formation of the macrocycle is characterized by the disappearance of these intense vibrations. The macrocycles **6**, **8** and **9** carrying one carboxy group exhibited characteristic vibrations of this group in IR spectra around 1710 cm^{-1}, although they were relatively weak.

NMR analysis of AzaPc and Pc is often complicated by dimerization of the planar molecules with subsequent strong broadening of all signals and undetectable aromatic carbons and hydrogens [4,25,32]. In our case, pyridine as the solvent together with the presence of peripheral bulky substituents suppressed sufficiently the dimerization and it allowed good NMR analysis. The structure of compounds **6**, **8** and **9** is unsymmetrical, and the macrocycle should theoretically yield 24 signals in the aromatic region of ^{13}C NMR spectra. Nonetheless, the differences between the signals of corresponding carbons in each quarter (A) are expected to be very low due to big distances from the point causing the asymmetry (quarter Z). That is perhaps why we found only 19–22 aromatic signals in ^{13}C NMR spectra. Some of the carbons may appear with the same chemical shifts. Some of the signals are also most probably overlapped with strong signals of traces of non-deuterated pyridine present in pyridine-d_5. The differences arising from unsymmetrical structure are observable also in the aliphatic region. Both carbons of *tert*-butylsulfanyl substituent gave three to five signals in expected area. Also ^{1}H NMR signals of *tert*-butyl hydrogens (expected to form a singlet) were split giving a multiplet, usually composed of three to four different singlets. Integration of these multiplets gave the calculated amounts of hydrogens. The ^{13}C NMR signal around 175 ppm attributed to carbonyl carbon was always found.

Mass spectra of compounds **8** and **9** were obtained in MALDI-TOF and confirmed the proposed structures giving the characteristic molecular ion peaks at corresponding masses $[M + H^+]$ (see Section 2). The structure was confirmed further by observing the fragments of the molecule. As we have shown before [25,32], the *tert*-butylsulfanyl groups are characteristically cleaved releasing isobutene and the fragment of the parent molecule at $m/z = [M - 56]$. Such fragmentation was observed in all cases. The MALDI-TOF technique required presence of very strong acid (trifluoroacetic acid) to support ionization. Magnesium complexes generally are not stable under such acidic conditions. Therefore, the mass spectra for compound **6** were obtained using milder technique—the electrospray ionization (ESI). This technique confirmed the mass of **6** and fragments corresponding to all seven (and only seven) released isobutenes were observed.

UV–vis spectra of AzaPc **6**, **8** and **9** are characterized by two main absorption bands—B-band around 370 nm and the Q-band around 655 nm arising from π–π* transition (Fig. 1). Conjugation of macrocyclic system with the free electron pair on heteroatom connecting peripheral chain is known to induce a bathochromic shift of the Q-band. The Q-bands of all prepared AzaPc are therefore red-shifted for approx. 20 nm compared to unsubstituted tetrapyrazinoporphyrazines (TPP) (635 nm in DMSO [33]) or TPP with peripheral chains bound through a carbon atom (636 nm in acetone [34]). The spectra of Mg (**6**) and Zn (**8**) complexes of the same macrocycle showed only negligible differences.

The shape of the Q-band of AzaPc **6**, **8** and **9** compared to symmetrical **10** or **11** is slightly distorted as a consequence of unsymmetrical distribution of electrons in the macrocycle. A small maximum around 626 nm that could be well observed in the spectrum of **11** is only a shoulder in the spectrum of unsymmetrical AzaPc **9** and it disappears completely in the spectra of **6** and **8** (Fig. 1 – inset). The extinction coefficients in the Q-band area decrease in the order **11** > **9** > **6**–**8** (Fig. 1). The sharp Q-band observed for symmetrical **11** became wider

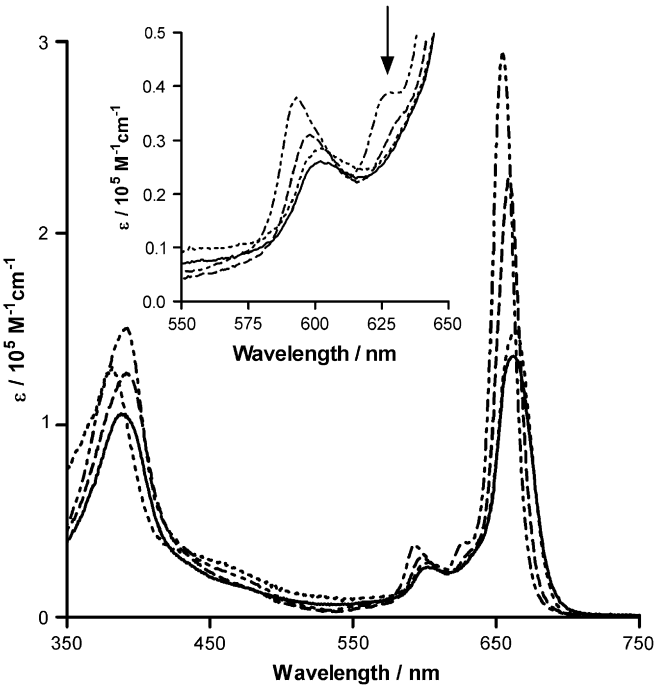


Fig. 1. UV–vis absorption spectra of AzaPc **6** (dotted), **8** (full), **9** (dashed) and **11** (dash-dotted) in DMF. Concentration 1.0×10^{-6} M.

and flatter for the unsymmetrical AzaPc. A small shoulder (or plateau) in the area of 440–480 nm arises from the n–π* transition of the lone pair electron on sulfur atoms [32,35] and is observable in the spectra of all compounds.

3.3. Singlet oxygen production

Singlet oxygen production is an important characteristic of potential photosensitizers. Therefore, we measured Φ_Δ values of the prepared compounds to evaluate their potential in PDT. The measurements of singlet oxygen were performed using a specific chemical trap—1,3-diphenylisobenzofuran (DPBF) [25]. DPBF reacts fast and specifically with singlet oxygen [36] by cycloaddition on the furan ring with subsequent opening of this ring. The final product 1,2-dibenzoylbenzene does not absorb anymore at 417 nm (λ_{max} of DPBF), hence the progress of the reaction can simply be monitored by observing the decrease of the absorption at this wavelength.

The Φ_Δ values for AzaPc **6**, **8** and **9** were found to be 0.07, 0.11 and 0.36, respectively. Recently we have discovered [37] that the energy from magnesium complexes of AzaPc in the excited state is released rather through photon emission than through photoreactions. The situation is reversed for zinc complexes of the same macrocycle. Therefore, considering the central metals, the zinc complexes are generally characterized by the highest singlet oxygen production and they are the most suitable for photodynamic application. The lower value for Mg complex **6** ($\Phi_\Delta = 0.07$) with respect to Zn complex **8** ($\Phi_\Delta = 0.11$) was therefore expected. We have shown previously [25,27] that the presence of nitrogen as a linking heteroatom between macrocycle and the peripheral alkyl chain decreases singlet oxygen production of AzaPc. Therefore it was not surprising that compound **8** produced a lower amount of singlet oxygen ($\Phi_\Delta = 0.11$) than compound **9** ($\Phi_\Delta = 0.36$). Nonetheless, other factors must be involved in the decrease of the Φ_Δ value of **8**, since the decrease is too big to be caused by the presence of one nitrogen atom in peripheral chain. For comparison, Φ_Δ of compound **11** with no nitrogens reached value of 0.66 [4]. We attribute this behavior to unsymmetrical distribution of electrons in macrocyclic system that may cause the changes in energy transformation (e.g. triplet state quantum yields or triplet state lifetimes).

One of the important factors influencing Φ_Δ values is also aggregation of AzaPc macrocycles. The absorbed energy of dimers or higher aggregates is released usually in the form of heat. This can be also used for anti-tumor therapy in application called photothermal sensitization [38]. However, the presence of six (**9**) or seven (**6**, **8**) bulky *tert*-butyls efficiently inhibits aggregation, and no dimers of AzaPc were detected in DMF solution used for singlet oxygen measurements. No differences in aggregation were observed between compounds bearing six or seven bulky substituents in this study. Therefore this reason must be excluded from the factors influencing the experimental values.

The value $\Phi_\Delta = 0.36$ for **9** is sufficient for the formation of an efficient amount of singlet oxygen. Some photosensitizers already approved for clinical practice have similar or even lower Φ_Δ values – e.g. for porfimer sodium (Photofrin®) $\Phi_\Delta = 0.28$ in PB [39], for temoporfin (Foscan®) $\Phi_\Delta = 0.30$ in ethanol or 0.31 in PBS with 10% of FCS [40], for talaporfin (Laserphyrin®) $\Phi_\Delta = 0.47$ in PBS [39], for protoporphyrin (a photosensitizer formed from aminolevulinic acid) $\Phi_\Delta = 0.36$–0.40 in Tris–HCl buffer [41].

4. Conclusions

Due to its good photochemical properties, red shift of the Q-band and excellent solubility in almost all common laboratory solvents, **9** offers promise as a component of third generation photosensitizers. This efficient photosensitizer, when combined with a targeting moiety, may enable the selective destruction of a targeted area by singlet oxygen formed after irradiation.

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