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Synthesis and studies on photodynamic activity of new water-soluble azaphthalocyanines

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Abstract

Aza analogues of phthalocyanines (AzaPc's) bearing four long chains with carboxy groups at the end and four "bulky" diethylamino groups on periphery were synthesised and characterised. Their sodium salts are very soluble in water. The first studies on photodynamic activity of this tetrapyrazinoporphyrazines (a type of AzaPc) are presented. The dye-sensitised photooxidation of 1,3-diphenylisobenzofurane via $^1O_2$ was studied in pyridine. Their photodynamic activity in vitro was not detected due to the aggregation behaviour of these compounds in water.

Keywords: Azaphthalocyanines; Tetrapyrazinoporphyrazines; Photodynamic therapy; Singlet oxygen

1. Introduction

Aza analogues of phthalocyanines (AzaPc's) can be used as industrial dyes and pigments, for controlling growth of microorganisms [1], as electrocatalysts for oxygen reduction [2], materials for electrochromic displays [3] and medias for optical storage [4]. As they are very similar to the phthalocyanines (Pc's), they can be used also in very similar applications as Pc. Among all the possible applications for Pc's (e.g. liquid crystals [5], chemical sensors [6], non-linear optics [7]) there is one that is the most important in medicine. They are known to be good photosensitisers (PS) and some of them are suitable agents for photodynamic therapy (PDT) [8,9].

PDT is a medical treatment, which employs the combination of a light and PS. After PS administration, it is cumulated in a neoplastic tissue and then illuminated by the light of the proper wavelength. It is followed by a singlet oxygen production by energy transfer from activated PS. Surrounding biomolecules are damaged and it starts a cascade of the biological response leading to a tumour death [10].

The first generation of PS (e.g. haematoporphyrin derivatives) absorbs the light at a relatively short wavelength (630 nm) and the penetration depth of the short-wavelength light in tissue is relatively shallow. The second generation of PS (e.g. Pc's, tetraphenylporphyrins, texaphyrins) absorbs at longer wavelengths [11]. The third generation is composed by the second generation PS conjugated to the small biomolecules (lipids, peptides, nucleosides, nucleotides) to enhance the selectivity of a dye cumulation [11].

Pc's and also AzaPc's possess one undesirable property. Owing to the extended π system, these macrocyclic compounds exhibit a high aggregation tendency forming dimeric and oligomeric species in solution [12,13]. This causes insolubility, hinders purification and characterisation of the compounds and precludes their use in PDT. The introduction of either long chains or bulky substituents to the periphery of the macrocycle should prevent the aggregation [14,15].

Since till this time there were no investigations on photodynamic activity of tetrapyrazinoporphyrazines (a type of AzaPc) we focused on this group of compounds. The aim of this work was to prepare water-soluble AzaPc (**5**–**7**). The long chain substituent (6-aminohexanoic acid) carries carboxy group that enables solubility in water (as a sodium salt) and is also suitable for a conjugation to the biomolecules. The second, bulky, substituent (diethylamine) was chosen to prevent the aggregation even more. Designing a substituent structure, we considered not only an aggregation behaviour but also lipophilicity. It was found that Pc with four carboxy groups is more photodynamically active than

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with eight carboxy groups or, on the other hand, completely hydrophobic [16]. These are two reasons why we chose the above-mentioned substituents. Photodynamic activity depends also on introduced central metal. Metal-free Pc does not exert any photodynamic activity. Diamagnetic central metals, such as Zn or Mg enhance phototoxicity of Pc's and therefore their potential for PDT [9]. Since PDT activity is mainly based on singlet oxygen, its production was determined by the dye-sensitised photooxidation of 1,3-diphenylisobenzofuran (DPBF), a specific scavenger of this toxic species [17].

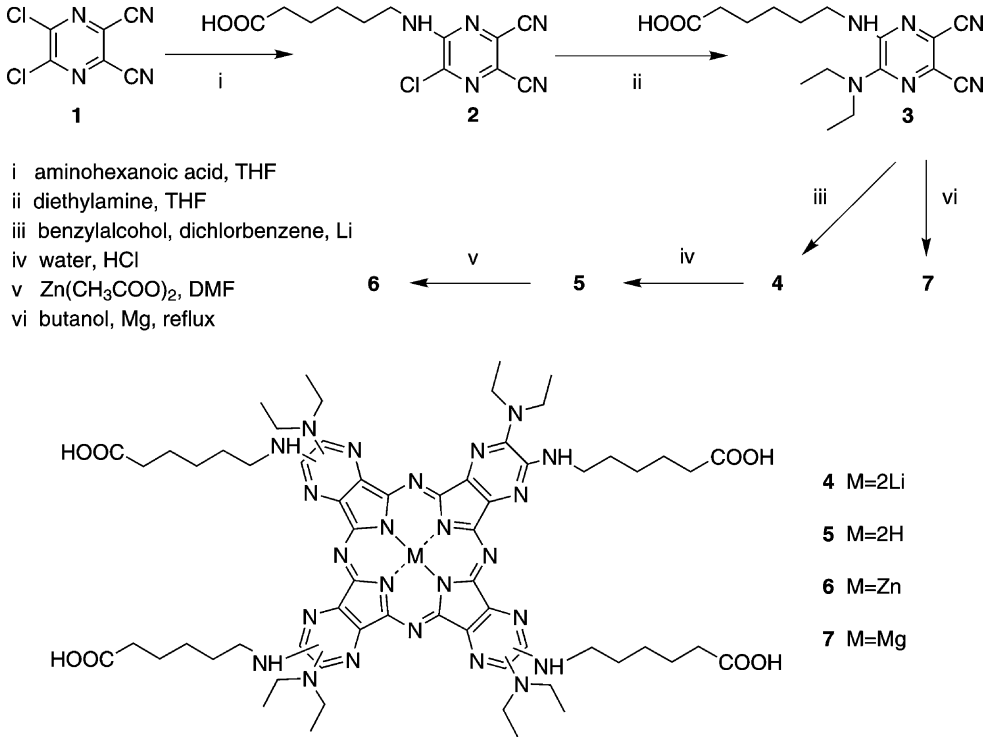
2. Results and discussion

Compound **1** was synthesised according to the literature [18]. This substance very easily undergoes nucleophilic substitution with primary and secondary amines to produce **2** in the first step and then **3** (Scheme 1). A synthesis of **4** was performed in benzylalcohol and 1,2-dichlorbenzene at 170 °C. This reaction proceeded very fast and the mixture turned dark green almost immediately after the addition of lithium. Dilithium Pc's (or AzaPc's) are labile toward water and acid and can easily be converted to metal-free Pc (AzaPc) [19] that can be accompanied by change of colour. Thus, treating **4** in water with HCl **5** was obtained. Purification of **5** seemed to be easy while it was sharply moving on TLC (chloroform/methanol 9:1). However, **5** was very strongly absorbed to silica in a column and could not be separated by a usual procedure. We took advantage of this feature and after our product was absorbed very strongly, we used methanol as an eluent and impurities were washed out from the column. The product was extracted from silica with 10% aqueous $NaHCO_3$.

ZnPc can be readily prepared by a metal/metal exchange from dilithium Pc [20] or by a metal insertion into metal-free Pc [21,22]. Since **4** precipitated from the reaction mixture in the form of lithium salt, the latter method had to be used. During insertion of Zn into **5** by zinc acetate, zinc salt of **6** originated. This salt is insoluble in all the solvents that we tried, even in DMF or hot water. However, after washing the compound with diluted HCl, free carboxy groups are released. TLC (chloroform/methanol 9:1) showed the reaction to be completed and all AzaPc contained Zn as the central metal.

The preparation of **7** was carried out in butanol with magnesium butoxide. Following procedure is similar to purification of **5**. Central Mg is also labile toward acids but not so much as Li. Nevertheless, during purification no acid stronger than acetic acid could be used to release free carboxy group, otherwise Mg is removed. On the other hand, acetic acid is probably not strong enough and some carboxy groups were still dissociated. This is probably the reason for a slightly less satisfactory CHN analysis of **5**. The amount of all the elements was found to be lower due to the presence of remaining Na (from $NaHCO_3$ used during purification).

All compounds **4**–**7** probably consist of four positional isomers, however, they have not been detected by any analytical method. Singlet oxygen measurement was carried out by a DPBF decomposition reaction in pyridine [16,17].



Scheme 1.

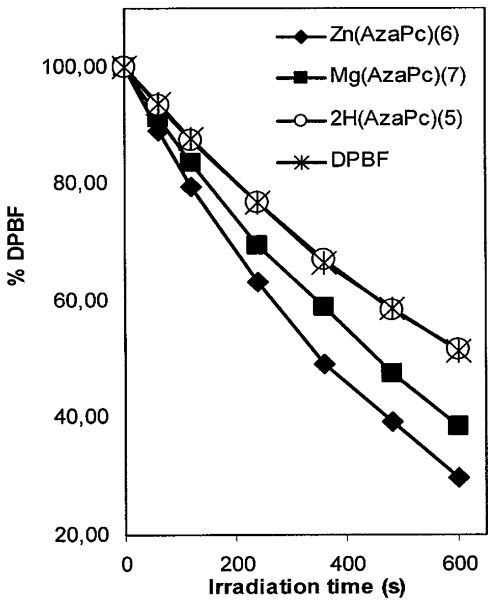


Fig. 1. Degradation of DPBF by singlet oxygen generation.

Since we did not use any filters, in absence of dyes DPBF was decomposed too. The rate of the decomposition was measured by the decrease of absorbance at 417 nm. It can be seen in Fig. 1 that in the presence of metal-free **5**, DPBF decomposes at the same rate as without any AzaPc. On the other hand, in the presence of **6** or **7**, the rate of the DPBF decomposition is higher, thus suggesting they release singlet oxygen after illumination. It is in a relation with findings for Pc's, where metal-free Pc does not exert any photodynamic activity while ZnPc belongs to the most potent photosensitisers [9].

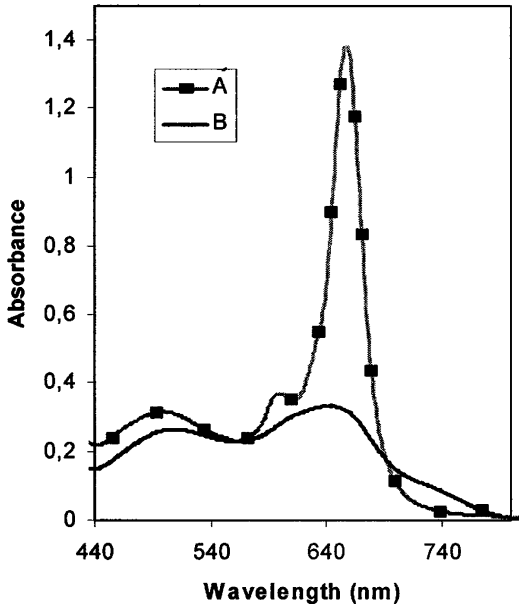


Fig. 2. Absorption spectra of **6** in pyridine (A) and in 0.2% aqueous $NaHCO_3$ (B). Concentration was 12.5×10^{-6} mol/dm^3 in both cases.

Tests on cells, surprisingly, did not show any photodynamic activity. It can probably be due to the aggregation in water. These aggregates can be detected on UV-Vis spectra (Fig. 2). While UV-Vis spectra in pyridine show sharp absorption peaks in the Q-band region the spectra in 0.2% aqueous $NaHCO_3$ show broadening and significant decrease of extinction coefficients due to the presence of the aggregates.

3. Conclusion

We confirmed our hypothesis about photodynamic activity of AzaPc's **5**–**7**. The dependence of activity on introduced central metal is the same as for Pc's and it encourages us in next synthesis of this type of compounds. Sodium salts of all above-mentioned AzaPc are well soluble in water. However, their aggregation behaviour in water, in spite of the bulky substituents and the long chains, precludes their use in photodynamic therapy directly. On the other hand, ZnAzaPc **6** is a promising compound for conjugation to the small biomolecules and thus for "the third generation of photosensitisers". The small biomolecules should prevent the aggregation completely.

4. Experimental section

4.1. Chemical part

All organic solvents used for the synthesis were of analytical grade. 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 Kofler block BOËTIUS PHMK 05 (VEB KOMBINAT NAGEMA, VEB Wagetechnik RAPIDO, Radebeul, Germany). Elementary analysis was carried out on Automatic Microanalysers EA1110CE (Fisons 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 and 75.43 MHz ^{13}C) Bruker Co. (Karlsruhe, Germany). Chemical shifts reported are given relative to internal $Si(CH_3)_4$. NMR signals of **6** and **7** showed broadening. UV-Vis spectra were recorded on spectrophotometer UV-2401PC, Shimadzu Europa GmbH (Duisburg, Germany).

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

Was prepared according to the literature [18].

4.1.2. 6-(3-Chloro-5,6-dicyano-pyrazin-2-ylamino)-hexanoic acid (*2*)

A total of 1.20 g (6 mmol) of **1** was dissolved in 60 ml of THF and 2.40 g (18 mmol) of fine powdered 6-amino-hexanoic acid was added. The suspension was stirred at room temperature for 12 h. Then unreacted 6-aminohexanoic

acid and its hydrochloride were removed by filtration, filtrate was evaporated, chromatographed on silica (hexane/ethylacetate/acetic acid 6:3:1) and recrystallised from ethanol/water. Yield 1.60 g of white-yellow needles (90%), mp 129–130 °C. IR (KBr): 3343, 2941, 2869, 2236 (s), 1707 (s), 1597 (s), 1233, 1131, 1081, 647. Anal. Calc. ($C_{12}H_{12}ClN_5O_2$): C 49.07, H 4.12, N 23.84. Found: C 49.11, H 4.28, N 23.82. ^{1}H NMR ($(CD_3)_2CO$) δ 8.06 (bs, 1H, NH), 3.60 (q, 2H, J = 7.1 Hz, CH_2), 2.28 (t, 2H, J = 7.1 Hz, CH_2), 1.78–1.55 (m, 4H, CH_2), 1.50–1.34 (m, 2H, CH_2). ^{13}C NMR ($(CD_3)_2CO$) Δ 174.8, 152.6, 138.7, 132.1, 117.6, 114.9, 114.6, 42.4, 34.0, 28.6, 26.8, 25.1.

*4.1.3. 6-(5,6-Dicyano-3-diethylamino-pyrazin-2-ylamino)-hexanoic acid (**3**)*

A total of 1.48 g (4.5 mmol) of **2** was dissolved in 60 ml of THF, cooled to 10 °C and 1.00 g (13.5 mmol) of diethylamine was added dropwise with stirring. A precipitate appeared immediately. The mixture was then refluxed for 3 h and the precipitate was filtered off. A filtrate was evaporated, chromatographed on silica (hexane/ethylacetate/acetic acid 6:3:1) and recrystallised from ethanol/water. Yield 1.50 g (90%) of yellow-white needles, mp 107–108 °C. IR (KBr): 3408, 2978, 2939, 2871, 2227 (s), 1706 (s), 1558 (s), 1532, 1504, 1432, 1230, 1146, 1078. Anal. Calc. ($C_{16}H_{22}N_6O_2$): C 58.17, H 6.71, N 25.44. Found: C 58.37, H 6.87, N 25.52. ^{1}H NMR (DMSO) Δ 11.98 (bs, 1H, OH), 7.58 (t, 1H, J = 5.5 Hz, NH), 3.46–3.29 (m, 6H, CH_2), 2.19 (t, 2H, J = 7.1 Hz, CH_2), 1.64–1.44 (m, 4H, CH_2), 1.37–1.23 (m, 2H, CH_2), 1.01 (t, 6H, J = 7.1 Hz, CH_3). ^{13}C NMR (DMSO) Δ 174.7, 147.5, 147.1, 122.8, 116.8, 116.1, 115.8, 43.4, 41.1, 33.8, 27.8, 26.3, 24.4, 12.6.

*4.1.4. {29H,31H-[2,9,16,23-Tetrakis(5-carboxy-pentylamino)-3,10,17,24-tetrakis(diethylamino)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato)] (2-)-N^{29},N^{30},N^{31},N^{32}}2H (**5**)*

One gram (3 mmol) of **3** was dissolved in the mixture of 2 ml of 1,2-dichlorbenzene and 2 ml of benzylalcohol. The reaction mixture was quickly heated to 170 °C and 0.15 g (21 mmol) of lithium was added. The mixture turned dark green immediately and a precipitate was formed. Heating was stopped in 10 min and acetone was added. The green-black precipitate was filtered, thoroughly washed with acetone, dissolved in a small amount of methanol and precipitated by ethylacetate. A product was then dissolved in 10% aqueous $NaHCO_3$ (solution turned deep purple), precipitated by few drops of conc. hydrochloric acid, filtered and washed with water. The crude purple product was then absorbed on silica and in a column (100 g of silica) washed very slowly with chloroform/methanol (9:1). After all **5** was strongly absorbed and no purple colour was moving ahead, the column was washed with methanol (until colourless) and dried. The pure compound **5** was extracted from silica with 10% aqueous $NaHCO_3$, precipitated with HCl, filtered, washed with water and dried. The product was finally recrystallised from acetone/ether. Yield: 360 mg (36%) of black-purple solid, mp >300 °C. IR (KBr): 3424, 2965, 2934, 2869, 1719 (s), 1640, 1530, 1475, 1146, 744. Anal. Calc. ($C_{64}H_{90}N_{24}O_8 + 3H_2O$): C 55.80, H 7.02, N 24.40. Found: C 55.67, H 7.15, N 24.54. UV-Vis (pyridine): λ_{max} (ε) 681 (76 000), 652 (58 000),. 503 (50 000), 365 (109 000). ^{1}H NMR (pyridine) δ 7.46–7.30 (m, 4H, NH), 4.21–4.03 (m, 8H, N–CH_2), 3.68–3.53 (m, 16H, N–CH_2), 2.75–2.59 (m, 8H, CH_2), 2.11–1.89 (m, 16H, CH_2), 1.86–1.67 (m, 8H, CH_2), 1.25–1.06 (m, 24H, CH_3). ^{13}C NMR (pyridine) δ 175.4, 151.7, 151.6, 151.6, 149.8, 44.0, 41.4, 34.3, 29.0, 26.8, 24.9, 12.4.

*4.1.5. {29H,31H-[2,9,16,23-Tetrakis(5-carboxy-pentylamino)-3,10,17,24-tetrakis(diethylamino)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato)] (2-)-N^{29},N^{30},N^{31},N^{32}}zinc(II) (**6**)*

Ninety-nine milligram (75 μmol) of **5** was dissolved in 5 ml of dry dimethylformamide, 164 mg (750 μmol) of dry zinc acetate was added and a mixture was stirred at 120 °C for 8 h. After cooling the reaction mixture methanol was added. Blue precipitate was collected by filtration and thoroughly washed with methanol, THF, diethylether and hot water. Then the precipitate was washed with 10% HCl, dissolved in 10% aqueous $NaHCO_3$, and precipitated with few drops of conc. HCl. The pure product was washed with water, dried and recrystallised from acetone/diethylether. Yield 60 mg (58%) of black-blue solid, mp >300 °C. IR (KBr): 3405, 2966, 2934, 2869, 1716 (s), 1641, 1534, 1478, 1253, 1147, 747. Anal. Calc. ($C_{64}H_{88}N_{24}O_8Zn + 3H_2O$): C 53.35, H 6.58, N 23.33. Found: C 53.05, H 6.49, N 23.56. UV-Vis (pyridine): λ_{max} (ε) 658 (110 000), 600 (29 000), 499 (25 000), 378 (121 000). ^{1}H NMR (pyridine) δ 7.18–7.04 (m, 4H, NH), 4.20–3.98 (m, 8H, N–CH_2), 3.62–3.48 (m, 16H, N–CH_2), 2.75–2.57 (m, 8H, CH_2), 2.07–1.87 (m, 16H, CH_2), 1.85–1.63 (m, 8H, CH_2), 1.22–1.02 (m, 24H, CH_3). ^{13}C NMR (pyridine) δ 176.0, 151.9, 151.7, 150.5, 146.6, 139.1, 139.0, 44.7, 41.9, 34.9, 29.7, 27.4, 25.3, 13.1.

*4.1.6. {29H,31H-[2,9,16,23-Tetrakis(5-carboxy-pentylamino)-3,10,17,24-tetrakis(diethylamino)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato)] (2-)-N^{29},N^{30},N^{31},N^{32}} magnesium(II) (**7**)*

Two hundred and forty milligram (10 mmol) of magnesium and a small crystal of iodine were refluxed in dry butanol for 7 h. Five hundred milligram (1.5 mmol) of **3** was then added and heated under reflux for another 3 h. Precipitated magnesium salt of **7** was filtered and washed with acetone, THF and chloroform. A product was then dissolved in glacial acetic acid and stirred at room temperature for 0.5 h, evaporated and thoroughly

washed with water and ethylacetate. This crude blue product was then purified by column chromatography similar to **5**. Yield 67 mg (13%) of black-blue solid, mp >300 °C. Anal. Calc. ($C_{64}H_{88}N_{24}O_8Mg + 3H_2O$): C 54.91, H 6.77, N 24.01. Found: C 54.46, H 6.66, N 23.08. UV-Vis (pyridine): λ_{max} (ε) 659 (111 000), 599 (25 000), 490 (21 000), 376 (106 000). IR (KBr): 3420, 2965, 2934, 1717 (s), 1641, 1534, 1477, 1254, 1146, 751. ^{1}H NMR (pyridine) δ 7.06 (bs, 4H, NH), 4.40–3.35 (m, 24H, NCH_2), 2.88–2.42 (m 8H, CH_2), 2.26–1.58 (m, 24H, CH_2), 1.55–0.79 (m, 24H, CH_3). ^{13}C NMR (pyridine) δ 175.3, 151.3, 146.4, 138.7, 44.2, 41.4, 34.3, 29.1, 26.7, 24.9, 12.6.

4.2. DPBF test

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

4.3. Biological part

Both toxicity and photodynamic activity of the AzaPc's **5**–**7** were tested in concentrations 10, 1 and 0.1 μM on the stabilised cell lines HL 60, Hep 2 and Hep G2 and on the protozoa culture of *Paramecium caudatum*. Cell lines were proceeded in microtitre wells in 2 ml of appropriate medium. Eighteen hours after, the dye was added in 0.2% aqueous $NaHCO_3$. Six hours after addition, cells were illuminated by the light source mentioned above from distance 0.5 m at the temperature 36 °C. Growth curve, morphology of cells and percentage of died cells were measured every 12 h till the time 72 h. Neither toxicity nor photodynamic activity were detected.

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References

[1] G. Reinert, G. Holzle, G. Graf, GB Patent 2,159,516 A (1985); G. Reinert, G. Holzle, G. Graf, Chem. Abstr. 105 (1986) 25830.
[2] A.A. Tanaka, C. Fierro, D.A. Sherson, E. Yaenger, Mater. Chem. Phys. 22 (3/4) (1989) 431.
[3] M. Yamana, N. Kashiwazaki, M. Yamamoto, T. Nakano, Jpn. J. Appl. Phys. Part 2 26 (7) (1987) L1113.
[4] T. Murayama, S. Maeda, Y. Kurose, Japan Patent 61,291,187 (1987); T. Murayama, S. Maeda, Y. Kurose, Chem. Abstr. 107 (1987) 68274.
[5] J. Simon, Nuov. J. Chim. 9 (1985) 159.
[6] D. Wöhrle, M. Kaneko, Yuki Gosei Kagaku Kyokai Shi 45 (1987) 837.
[7] A. Grund, A. Kaltbeitzel, A. Mathy, R. Schwarz, C. Bubeck, P. Vernmehren, M. Hanack, J. Phys. Chem. 96 (1992) 7450.
[8] D.J. Spikes, Photochem. Photobiol. 43 (1986) 691.
[9] I. Rosenthal, Photochem. Photobiol. 53 (1991) 859.
[10] B.W. Henderson, T.J. Dougherty, Photochem. Photobiol. 55 (1992) 145.
[11] E.D. Sternberg, D. Dolphin, C. Brückner, Tetrahedron 54 (1998) 4151.
[12] E.A. Lukyanets, J. Porphyrins Phthalocyan. 3 (1999) 424.
[13] H. Ali, J.E. van Lier, Chem. Rev. 99 (1999) 2379.
[14] M.T.M. Choi, P.P.S. Li, D.K.P. Ng, Tetrahedron 56 (2000) 3881.
[15] S. Tai, N. Hayashi, J. Chem. Soc., Perkin Trans. 2 (1991) 1275.
[16] K. Oda, S. Ogura, I. Okura, J. Photochem. Photobiol. B Biol. 59 (2000) 20.
[17] U. Michelsen, H. Kliesch, G. Schnurpfeil, A.K. Sobbi, D. Wöhrle, Photochem. Photobiol. 64 (4) (1996) 694.
[18] E.H. Mørkved, L.T. Holmaas, H. Kjøsen, G. Hvistendahl, Acta Chem. Scand. 50 (1996) 1153.
[19] D.S. Terekhov, K.L.M. Nolan, C.R. McArthur, C.C. Leznoff, J. Org. Chem. 61 (1996) 3034.
[20] M.J. Cook, A.J. Dunn, S.D. Howe, A.J. Thomson, K.J. Harrison, J. Chem. Soc., Perkin Trans. 1 (1988) 2453.
[21] H. Kliesch, A. Weitemeyer, A. Müller, D. Wöhrle, Liebigs Ann. (1995) 1269.
[22] C.C. Leznoff, M. Hu, C.R. McArthur, Y. Qin, J.E. van Lier, Can. J. Chem. 72 (1994) 1990.