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Magnesium Azaphthalocyanines: An Emerging Family of Excellent Red-Emitting Fluorophores

Petr Zimcik,*[,†] Veronika Novakova,[‡] Kamil Kopecky,[†] Miroslav Miletin,[†] Rabia Zeynep Uslu Kobak,[§] Eva Svandrlikova,[†] Lenka Váchová,[†] and Kamil Lang[∥]

[†]Department of Pharmaceutical Chemistry and Drug Control, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Heyrovskeho 1203, 50005, Hradec Kralove, Czech Republic

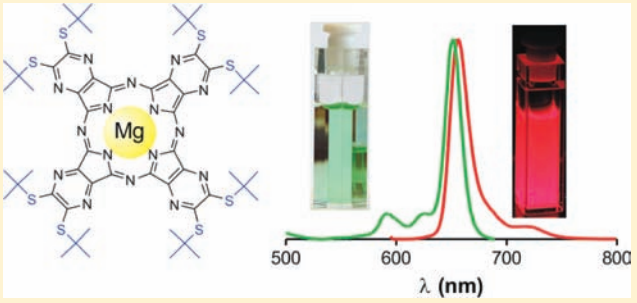
[‡]Department of Biophysics and Physical Chemistry, Faculty of Pharmacy in Hradec Kralove, Charles University in Prague, Heyrovskeho 1203, 50005, Hradec Kralove, Czech Republic

[§]Department of Chemistry, Faculty of Arts and Sciences, Technical University of Istanbul, TR34469 Maslak, Istanbul, Turkey

[∥]Institute of Inorganic Chemistry, Academy of Sciences of the Czech Republic, v.v.i., Husinec-Řež 1001, 250 68 Řež, Czech Republic

Supporting Information

ABSTRACT: Magnesium(II), zinc(II), and metal-free phthalocyanines (Pcs) and azaphthalocyanines (AzaPcs) containing alkylsulfanyl, aryloxy, and dialkylamino peripheral substituents have been synthesized. The complexation of magnesium(II) by metal-free Pcs and AzaPcs has been studied in detail to determine the optimal reaction conditions necessary to ensure a complete conversion. Photophysical and photochemical measurements in tetrahydrofuran showed that magnesium(II) AzaPcs with aryloxy and alkylsulfanyl substituents have excellent fluorescent properties (Φ_F up to 0.73) and that the corresponding zinc(II) Pcs are efficient singlet oxygen producers (Φ_Δ up to 0.68). The presence of dialkylamino substituents causes intramolecular charge transfer within the molecule that competes with fluorescence and singlet oxygen formation. Alkylsulfanyl MgAzaPc and ZnAzaPc were the most photostable compounds among the series of studied derivatives. In addition, high molar absorption coefficients ($\varepsilon \sim 300\,000$ M^{-1} cm^{-1}), absorption ($\lambda_{max} \sim 650$ nm), and emission ($\lambda_{em} \sim 660$ nm, high Φ_F) in the red region suggest that these molecules are potential fluorescent probes that are superior to the commercial red cyanine dye Cy5. MgAzaPc, when incorporated into lipidic bilayers of liposomes, maintains excellent fluorescence properties ($\Phi_F = 0.64$). Water-soluble MgAzaPc with quaternary ammonium peripheral substituents retained a high fluorescence quantum yield even in water ($\Phi_F = 0.25$). The described properties show that magnesium(II) AzaPcs are excellent red-emitting fluorophores with potential applications as fluorescent probes in sensing or in vitro imaging applications.



INTRODUCTION

Phthalocyanines (Pcs) are planar macrocyclic synthetic dyes capable of complexing a wide variety of metals. Because of their stability as well as their chemical, photophysical, photochemical, electrochemical, and catalytic properties, Pcs have attracted the attention of researchers in many areas.[1] The aza analogs—azaphthalocyanines (AzaPcs)—have been investigated in similar applications; however, the presence of nitrogen atoms in the macrocycle structure may add new specific properties.[2−4] Recently, Pcs have found applications in fluorescence probing[5] because of their suitable absorption and emission in the red region of the visible spectrum. The use of red or near-infrared excitation and emission is important for biological applications because longer-wavelength light penetrates deeper into tissues, is less scattered, and the autofluorescence of endogenous chromophores is limited. The significance of red-emitting fluorophores has therefore increased in recent years, concurrently with the increased interest in in vivo imaging.[6]

The photophysical and photochemical properties of Pcs and their analogues are known to be significantly influenced by a central metal and peripheral substitution.[7] So far, the fluorescent probes based on Pcs exhibit relatively low fluorescence quantum yields (Φ_F) because of the presence of central zinc(II) cations. The presence of heavy metals leads to the increase of the probability of molecular relaxation via intersystem crossing,[8] which can cause high quantum yields of singlet oxygen formation (Φ_Δ) and low Φ_F.[7] The enhancement of the spin−orbit coupling in compounds substituted or complexed with heavy atoms is known as the heavy-atom effect.[9] While working with Pc and AzaPc, we noticed that several research groups have reported significant fluorescent properties of magnesium complexes of Pcs[10] and AzaPcs.[3,11] Magnesium(II) represents the lightest cation that is stable in

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the center of these macrocycles. Lithium(I), beryllium(II), and sodium(I) are only weakly bound and can be easily replaced by hydrogen atoms in weakly acidic media. Boron(III) forms subphthalocyanines that absorb and emit at lower wavelengths.[12] For these reasons, the magnesium(II) cation appears to be the metal of a choice for porphyrinoid compounds intended for fluorescence probing in the red region. Despite several reports having been published on MgPcs and MgAzaPcs, no structure−activity relationships with detailed discussion of the photophysical properties and photostability have thus far appeared.

In this work, we report the photophysical properties of magnesium(II) and zinc(II) Pcs and AzaPcs and their optimized structure with the respect to a peripheral substitution and a macrocyclic core. Both the Pc and AzaPc macrocycles with different heteroatoms that connect peripheral substituents, inhibit aggregation, and considerably affect the photophysical properties are introduced into the study. We also report here synthetic aspects that are important for the successful development of magnesium(II) derivatives.

EXPERIMENTAL SECTION

General Procedures. All organic solvents used for synthesis were of analytical grade. Anhydrous butanol was stored over magnesium and distilled prior to use. Anhydrous pyridine was dried over KOH, distilled, and stored over molecular sieves. Tetrahydrofuran for photophysical and photochemical measurements was of HPLC grade from Sigma-Aldrich. All chemicals for synthesis were obtained from established suppliers (Aldrich, Acros, Merck) and used as received. Zinc phthalocyanine (ZnPc) was purchased from Sigma-Aldrich. TLC was performed on Merck aluminum sheets coated with silica gel 60 F254. Merck Kieselgel 60 (0.040−0.063 mm) was used for column chromatography. Melting points were measured on an Electrothermal IA9200-series digital melting-point apparatus (Electrothermal Engineering, Southend-on-Sea, Essex, Great Britain). The infrared spectra were measured on a Nicolet 6700 spectrometer in ATR mode. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury Vx BB 300 NMR spectrometer. Chemical shifts are given relative to $Si(CH_3)_4$ and were locked to the signal of the solvent. Elemental analyses were performed on an Automatic Microanalyser EA1110CE (Fisons Instruments, Milan, Italy). The UV−vis spectra were recorded using a Shimadzu UV-2401PC spectrophotometer. The steady-state fluorescence spectra were measured using an AMINCO-Bowman Series 2 luminescence spectrometer. Fluorescence lifetime measurements were performed on a Fluorolog 3 spectrometer (Horiba JobinYvon) using laser-diode excitation at 405 nm (NanoLED-405LH, pulse width 750 ps, repetition rate 1 MHz). The emission was recorded at fluorescence maxima using a cooled TBX-05-C photon detection module in a time-correlated single-photon counting regime. The decay curves were fitted to exponential functions using the iterative reconvolution procedure of the DAS6 software (v. 6.4, Horiba JobinYvon, 2009). MALDI-TOF mass spectra were recorded in a positive reflectron mode on a Voyager-DE STR mass spectrometer (Applied Biosystems, Framingham, MA, USA) in *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]-malononitrile as a matrix. The instrument was calibrated externally with a five-point calibration using a Peptide Calibration Mix1 kit (LaserBio Laboratories, Sophia-Antipolis, France).

Syntheses. The compounds **1Mg**,[2] **1Zn**,[2] **1H**,[2] **2Zn**,[13] **3Mg**,[14] **3Zn**,[15] **3H**,[15] **4Mg**,[2] **4Zn**,[2] **4H**,[2] and **6**[16] were prepared according to published procedures. The new analytical data for these compounds are presented in the Supporting Information. All the samples used for singlet oxygen, fluorescence, and photobleaching quantum yield determinations were purified on silica thin-liquid chromatography (TLC) plates to ensure their high purity. The eluents used are given for each compound. The corresponding parts of the TLC plates were scraped out, and the compounds were extracted using THF.

*2,3,9,10,16,17,23,24-Octakis(2,6-diisopropylphenoxy)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato Magnesium(II) (**2Mg**).* Anhydrous magnesium acetate (71 mg, 0.50 mmol) and dried 5,6-bis(2,6-diisopropylphenoxy)pyrazine-2,3-dicarbonitrile[13] (300 mg, 0.62 mmol) were placed into a small flask, purged with argon, and immersed into a preheated oil bath (160 °C). Anhydrous quinoline (0.5 mL) (double distilled from calcium hydride) was added, and the solution was further heated under argon for 24 h. After cooling, the mixture was poured into a water/methanol 1:1 solution (150 mL), stirred for an hour at room temperature (rt), and the suspension was filtered. The crude product was purified on silica with chloroform/acetone 20:1 as an eluent. The pure fractions were evaporated and washed with methanol to obtain 128 mg (42%) of a blue-green solid. R_f (chloroform/acetone 20:1) = 0.53. UV−vis (DMF) λ/nm (ε/M^{-1} cm^{-1}): 627 (184 300), 571 (24 800), 364 (106 100). MS (MALDI-TOF) *m*/*z*: 1953.7 [M]$^+$, 1992.6 [M + K]$^+$. ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm = 151.4, 149.9, 147.9, 142.5, 141.1, 126.5, 124.4, 28.1, 23.5. ^{1}H NMR ($CDCl_3$, 300 MHz): δ/ppm = 7.59−7.51 (m, 8H, ArH), 7.42 (d, *J* = 7.6 Hz, 16H, ArH), 3.31 (hept, *J* = 7.3 Hz, 16H, CH), 1.28 (d, *J* = 6.9 Hz, 96H, CH_3). IR (ATR): 2964, 2870, 1546, 1467, 1398, 1294, 1247, 1212, 1160, 1145, 1110, 1093, 1056, 930, 867 cm^{-1}. Calcd. for $C_{120}H_{136}MgN_{16}O_8$ + $5H_2O$: C, 70.48; H, 7.20; N, 10.96%. Found: C, 70.45; H, 7.53; N, 11.03%.

*2,3,9,10,16,17,23,24-Octakis(2,6-diisopropylphenoxy)-1,4,8,11,15,18,22,25-(octaaza)phthalocyanine (**2H**).* This compound was prepared by a different procedure than that described in the literature.[17] The compound **2Mg** (71 mg, 36 μmol) was dissolved in chloroform (4 mL), and *p*-toluenesulfonic acid (68 mg, 395 μmol) dissolved in THF (2 mL) was added. The mixture was stirred for 3 h at rt, and the solvents were evaporated. The solid was washed with water and methanol and purified on a silica column with toluene/THF 50:1 as an eluent. The pure fractions were evaporated and washed with methanol to obtain 61 mg (87%) of a blue-green solid. R_f (toluene/THF 20:1) = 0.57. UV−vis (THF) λ/nm (ε/M^{-1} cm^{-1}): 646 (122 400), 609 (91 900), 558 (21 500), 349 (102 900). MS (MALDI-TOF) *m*/*z*: 1887.7 [M − *i*Pr]$^+$, 1930.7 [M]$^+$, 1953.7 [M + Na]$^+$, 1969.7 [M + K]$^+$. ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm = 152.2, 147.8, 141.0, 126.7, 124.4, 28.2, 23.5. ^{1}H NMR ($CDCl_3$, 300 MHz): δ/ppm = 7.58 (dd, *J* = 8.3, 7.0 Hz, 8H, ArH), 7.45 (d, *J* = 7.6 Hz, 16H, ArH), 3.30 (hept, *J* = 6.8 Hz, 16H, CH), 1.31 (d, *J* = 6.9 Hz, 96H, CH_3), −2.12 (s, 2H, pyrrole NH). IR (ATR): 3300 (NH), 2965, 2870, 1547, 1531, 1467, 1401, 1363, 1312, 1244, 1217, 1142, 1093, 1057, 1023, 920, 853 cm^{-1}. Calcd. for $C_{120}H_{138}N_{16}O_8$ + $2H_2O$: C, 73.22; H, 7.27; N, 11.38%. Found: C, 73.48; H, 7.65; N, 11.40%.

*2,3,9,10,16,17,23,24-Octakis(2,6-diisopropylphenoxy)-phthalocyaninato magnesium(II) (**5Mg**).* Magnesium turnings (168 mg, 7 mmol) and a small crystal of iodine were refluxed in anhydrous butanol (10 mL) for 3 h. 4,5-Bis(2,6-diisopropylphenoxy)-phthalonitrile[18] (480 mg, 1 mmol) was added to the formed magnesium butoxide in butanol and refluxed for 3 h. After cooling, the mixture was concentrated under reduced pressure, poured into water/methanol/acetic acid 5:5:1 (200 mL) and stirred for 30 min at rt. The crude product was filtered and successively washed with water and methanol. The product was subsequently adsorbed onto silica (3 g) and washed successively with methanol until a colorless wash solution was passed. The product on silica was dried and chromatographed on silica using a step gradient that started with toluene and was followed with toluene/THF 100:1. The pure fractions were evaporated and washed with methanol to obtain 150 mg (31%) of a bright-green solid. The product, both in solution and on silica, is highly sensitive to light; it was therefore protected from exposure to daylight during purification and all subsequent manipulations. R_f (toluene) = 0.47. UV−vis (DMF) λ/nm (ε/M^{-1} cm^{-1}): 677 (259 100), 647 (34 100), 611 (38 100), 362 (109 300). UV−vis (THF) λ/nm (ε/M^{-1} cm^{-1}): 678 (334 700), 647 (39 400), 611 (45 300), 360 (119 500). MS (MALDI-TOF) *m*/*z*: 1944.7 [M]$^+$. ^{13}C NMR ($CDCl_3$/pyridine-d_5, 75 MHz): δ/ppm = 152.6, 149.6, 148.5, 141.0, 132.1, 125.7, 124.1, 106.5, 26.7, 23.5, 22.2. ^{1}H NMR ($CDCl_3$/pyridine-d_5, 300 MHz): δ/ppm = 7.93 (s, 8H, core ArH), 7.31 (dd, *J* = 8.4, 6.9 Hz, 8H, ArH), 7.19 (d, *J* = 7.2 Hz, 16H), 3.20 (hept, *J* = 6.9 Hz, 16H,

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CH), 1.17−0.81 (m, 96H, CH_3). IR (ATR): 2964, 2930, 2870, 1611, 1585, 1483, 1440, 1397, 1363, 1344, 1328, 1268, 1183, 1138, 1087, 1044, 1025, 937, 896, 860 cm^{-1}. Calcd. for $C_{128}H_{144}MgN_8O_8 + 4H_2O$: C, 76.15; H, 7.59; N, 5.55%. Found: C, 75.83; H, 7.91; N, 5.35%.

*2,3,9,10,16,17,23,24-Octakis(2,6-diisopropylphenoxy)-phthalocyanine (**5H**).* The compound **5Mg** (58.4 mg, 30 μmol) was dissolved in chloroform (4 mL), and *p*-toluenesulfonic acid (57 mg, 300 μmol) dissolved in THF (2 mL) was added. The mixture was stirred for 90 min at rt, and the solvents were evaporated. The solid was washed with water and methanol and chromatographed on silica with toluene/hexane 1:1 as an eluent. The pure fractions were evaporated and washed with methanol to obtain 35 mg (60%) of a bright-green solid. The product, both in solution and on silica, is highly sensitive to light; it was therefore protected from exposure to daylight during purification and all subsequent manipulations. R_f (hexane/toluene 1:1) = 0.56. UV−vis (THF) λ/nm (ε/M^{-1} cm^{-1}): 702 (215 900), 664 (177 800), 647 (52 700), 636 (52 100), 601 (33 600), 418 (46 700), 350 (95 800). MS (MALDI-TOF) *m*/*z*: 1922.8 $[M]^+$. ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm = 151.0, 149.1, 146.0, 141.7, 130.8, 126.4, 124.7, 107.4, 27.4, 24.3, 23.0. ^1H NMR ($CDCl_3$, 300 MHz): δ/ppm = 8.14 (s, 8H, core ArH), 7.57 (dd, *J* = 8.6, 6.7 Hz, 8H, ArH), 7.49−7.44 (m, 16H, ArH), 3.44 (hept, *J* = 6.7 Hz, 16H, CH), 1.29 (broad s, 96H, CH_3), −0.84 (s, 2H, NH). IR (ATR): 3300 (NH), 2964, 2930, 2869, 1612, 1584, 1493, 1439, 1401, 1363, 1327, 1266, 1222, 1184, 1147, 1093, 1061, 1016, 937, 878, 849. Calcd. for $C_{128}H_{146}N_8O_8 + 1H_2O$: C, 79.14; H, 7.68; N, 5.77%. Found: C, 79.13; H, 7.99; N, 5.86%.

*2,3,9,10,16,17,23,24-Octakis(2,6-diisopropylphenoxy)-phthalocyaninato Zinc(II) (**5Zn**).* The compound was prepared using a procedure different from that described in the literature.[18] The compound **5H** (35 mg, 18 μmol) was dissolved in pyridine (15 mL), anhydrous zinc acetate (33 mg, 182 μmol) was added, and the mixture was refluxed for 30 min. The solvent was evaporated, the solid was washed with water and methanol, and the crude product was purified on silica with toluene/hexane 2:1 as an eluent. The pure fractions were evaporated and washed with methanol to obtain 36 mg (99%) of a bright-green solid. The product, both in solution and on silica, was highly sensitive to light; it was therefore protected from exposure to daylight during purification and all subsequent manipulations. R_f (hexane/toluene 1:1) = 0.05. UV−vis (DMF) λ/nm (ε/M^{-1} cm^{-1}): 676 (273 400), 647 (36 100), 610 (39 700), 362 (79 400). UV−vis (THF) λ/nm (ε/M^{-1} cm^{-1}): 676 (368 800), 645 (45 500), 610 (51 800), 356 (117 500). MS (MALDI-TOF) *m*/*z*: 1984.6 $[M]^+$. ^{13}C NMR ($CDCl_3$, 75 MHz): δ/ppm = 152.7, 150.6, 149.2, 141.8, 132.2, 126.3, 124.7, 107.4, 27.4, 24.2, 23.2. ^1H NMR ($CDCl_3$, 300 MHz): δ/ppm = 8.15 (s, 8H, core ArH), 7.56 (dd, *J* = 8.6, 6.6 Hz, 8H, ArH), 7.49−7.33 (m, 16H, ArH), 3.46 (hept, *J* = 6.9 Hz, 16H, CH), 1.46−1.14 (m, 96H, CH_3). IR (ATR): 2964, 2870, 1611, 1585, 1460, 1440, 1401, 1346, 1328, 1271, 1223, 1185, 1143, 1095, 1028, 937, 898, 860 cm^{-1}. Calcd. for $C_{128}H_{144}N_8O_8Zn + 3H_2O$: C, 75.29; H, 7.40; N, 5.49%. Found: C, 75.54; H, 7.71; N, 5.59%.

*2,3,9,10,16,17,23,24-Octakis(2-diethylaminoethylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato magnesium(II) (**7**).* Magnesium turnings (360 mg, 15 mmol) and a small crystal of iodine were refluxed in anhydrous butanol (10 mL) for 3 h. 5,6-Bis(2-diethylaminoethylsulfanyl)pyrazine-2,3-dicarbonitrile[16] (**6**) (196 mg, 0.5 mmol) was dissolved in anhydrous butanol (5 mL), added to the suspension of magnesium butoxide in butanol, and the mixture was refluxed for 3 h. After cooling, the solvent was evaporated. The residuals were then extracted with THF and filtered, and THF was evaporated. The product was adsorbed onto silica (2 g) and washed successively with methanol. The product on silica was subsequently dried and chromatographed with pyridine/methanol 4:1 as an eluent. The green fractions were combined and chromatographed on neutral alumina with chloroform/THF 1:1 followed with THF/methanol 10:1 as eluents to obtain 70 mg (30%) of a dark-green solid. R_f (pyridine/methanol 4:1) = 0.72. The product was isolated as a 5:1 mixture with the compound **7a**. UV−vis (DMF) λ/nm (ε/M^{-1} cm^{-1}): 654 (265 100), 593 (37 000), 382 (151 300). MS (MALDI-TOF) *m*/*z*: 1533.6 $[M - SCH_2CH_2N(CH_2CH_3)_2 + OC_4H_9$, compound **7a**$]^+$, 1564.6 $[M - C_2H_4]^+$, 1592.6 $[M]^+$. ^{13}C NMR (pyridine-d_5, 75 MHz): δ/ppm = 157.7, 151.0, 146.4, 51.9, 47.3, 29.8, 12.5. Due to a mixed composition and overlapping signals, the number of protons in the ^1H NMR spectrum is related to the well-separated methylene $O-CH_2CH_2CH_2CH_3$ at δ = 1.98 ppm. ^1H NMR (pyridine-d_5, 300 MHz): δ/ppm = 4.99−4.85 (broad, overlapped with H_2O residual signal, $O-CH_2$), 4.55−3.80 (m, 43H, $S-CH_2$), 3.40−3.03 (m, 43H, NCH_2), 3.02−2.47 (m, 86H, NCH_2CH_3), 2.06−1.91 (m, 1H, $O-CH_2CH_2$), 1.79−1.60 (m, 1H, $O-CH_2CH_2CH_2$), 1.54−0.76 (m, 132H, CH_3 + CH_3). IR (ATR): 2968, 2933, 2809, 1641, 1513, 1462, 1383, 1335, 1255, 1230, 1199, 1164, 1092, 1069, 973, 851 cm^{-1}.

*2,3,9,10,16,17,23,24-Octakis(2-(triethylammonio)ethylsulfanyl)-1,4,8,11,15,18,22,25-(octaaza)phthalocyaninato Magnesium(II) Octaiodide (**8**).* The synthesis of this compound was adopted from a published procedure.[19] Compound **7**, prepared as above (60 mg, 38 μmol, as a mixture with **7a**), was dissolved in ethyl iodide (10 mL), and the solution was stirred for 2 d at rt. The green precipitate, which appeared after 2 d, was dissolved by the addition of *N*-methylpyrrolidinone (10 mL), and the reaction was stirred for 5 d at rt. The green solution was poured into diethyl ether; the precipitate was collected and washed thoroughly with diethyl ether and acetone. The product was then dissolved in MeOH and filtered, and the solvent was evaporated. The product was recrystallized from methanol/diethyl ether to obtain 96 mg (90%) of a dark-green solid. The product was isolated as a mixture with **8a**. UV−vis (DMF) λ/nm (ε/M^{-1} cm^{-1}): 655 (171 200), 596 (24 900), 386 (106 600). UV−vis (water) λ/nm (ε/M^{-1} cm^{-1}): 652 (166 300), 593 (23 800), 382 (114 600), 224 (132 700). ^{13}C NMR (D_2O + dimethylsulfoxide-d_6, 75 MHz): δ/ppm =55.9, 53.5, 31.0, 7.8, aromatic signals were not detected. ^1H NMR (D_2O + dimethylsulfoxide-d_6, 300 MHz): δ/ppm = 4.45−4.27 (m, 16H, SCH_2), 3.90−3.73 (m, 16H, NCH_2), 3.70−3.52 (m, 48H, NCH_2), 2.36−2.14 (m, 72H, CH_3); all signals were broad. IR (ATR): 2978, 1658, 1519, 1452, 1396, 1334, 1309, 1250, 1178, 1108, 1093, 1027, 971, 852 cm^{-1}. Calcd. for $C_{88}H_{152}I_8MgN_{24}S_8 + 11H_2O$: C, 34.76; H, 5.77; N, 11.06%. Found: C, 34.75; H, 5.45; N, 10.68%.

Optimization Protocols for Complexation of Magnesium(II) by 1H. Compound **1H** (20 mg, 16.3 μmol) was dissolved in an appropriate solvent (5 mL) and a magnesium(II) salt (163 μmol) was added. The mixture was immersed in a preheated oil bath and stirred at the selected temperature. The progress of the reaction was monitored using TLC. The following different conditions were tested:

(a) The reaction was performed in anhydrous pyridine under reflux with different sources of anhydrous magnesium(II) cations (acetate, chloride, lactate, and carbonate salts, as well as magnesium oxide).

(b) The reaction was performed with anhydrous magnesium acetate at 120 °C in different anhydrous solvents (pyridine, DMF, DMSO, *N*,*N*-dimethylacetamide, and THF).

(c) The reaction was performed under reflux and different levels of anhydrous conditions were considered: anhydrous magnesium acetate, magnesium acetate tetrahydrate, anhydrous pyridine, and analytical-grade pyridine (that contained 0.1% water).

(d) The reaction was performed in THF with anhydrous magnesium acetate under reflux with increasing amount of pyridine: 2 (2.6 mg, 32.6 μmol), 10 (12.9 mg, 163 μmol), 100 (129 mg, 1.6 mmol), and 1000 equiv (1.29 g, 16 mmol).

(e) The reaction was performed in anhydrous pyridine with anhydrous magnesium acetate at different oil-bath temperatures (rt, 45, 70, 90 °C, and reflux).

General Procedure for Complexation of Magnesium(II) with Compounds 1H−5H. Metal-free Pc or AzaPc (**1H**−**5H**) (20 μmol) was dissolved in anhydrous pyridine (2.5 mL), and anhydrous magnesium acetate (28 mg, 200 μmol) was added. The mixture was heated to reflux and monitored using TLC. When the reaction was finished, the solvent was evaporated; the solid was then washed with water and methanol and dried. The compounds **3Mg** and **5Mg** were further purified on a silica column using the eluents previously described.

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Measurements of Quantum Yields. Quantum yields of singlet oxygen formation (Φ_Δ), fluorescence (Φ_F), and photodecomposition (Φ_{Ph}) were measured using the comparative methods with zinc phthalocyanine (ZnPc) as a reference ($\Phi_\Delta = 0.53$ in THF,[20] $\Phi_F = 0.30$ in chloronaphthalene,[21] and $\Phi_{Ph} = 2.23 \times 10^{-5}$ in DMF[22]) (see the Supporting Information for details).

Incorporation of 1Mg into Liposomes. Large unilamellar vesicles formed by the extrusion technique (LUVETs) were prepared from a suspension of multilamellar vesicles (MLVs).[23] The lipids (1,2-dioleoylphosphatidylcholine, DOPC, from Lipoid GmbH; 18.8 mg, 24 μmol) were dissolved in chloroform (10 mL), THF stock solution (200 μM) of **1Mg** was added (150 μL, 30 nmol), and the solvent was evaporated in a 100-mL round-bottom flask on a water bath at 37 °C. The thin lipid film was further left on a water bath for 30 min at 5 mbar to remove traces of organic solvents. Phosphate buffer saline (PBS, pH 7.4) was added (1 mL), and the lipids were removed from the flask walls by gentle hand-shaking. The suspension was then vortexed for 3 min to form MLVs and left for 3 h at rt to allow complete swelling. LUVETs were prepared from the MLV suspension using a LiposoFast Basic hand extruder (Avestin, Canada).[24] The suspension was passed back and forth 21 times through two stacked polycarbonate filters (pore diameter 100 nm) at rt and subsequently diluted to its final concentration using PBS. The final dye concentration was 30 μM with a lipid-to-dye ratio of 800.

RESULTS AND DISCUSSION

Compound Syntheses. The syntheses of the Pc and AzaPc derivatives (Figure 1) started from corresponding aromatic *ortho*-dicarbonitriles. The Linstead method,[25] which is based on alkoxides as initiators of cyclotetramerization, was used for the preparation of **1Mg**, **3H**, **3Mg**, **4Mg**, **4H**, and **5Mg**. The method affords high yields and can be used for the synthesis of Pc macrocycles. However, the low stability of some peripheral substituents under the Linstead conditions complicates the synthesis of aryloxy (compound **2Mg**) and some alkylsulfanyl (compound **7Mg**) AzaPcs. Therefore, the compound **2Mg** was synthesized by an alternative method using the template effect of magnesium(II) cations in a high-boiling solvent (see below). The synthesis of **7Mg** is discussed in detail in the following paragraph.

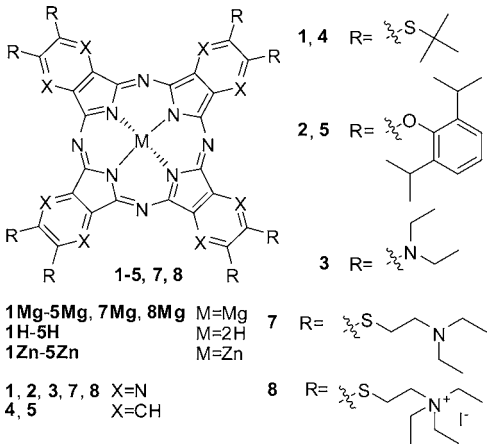
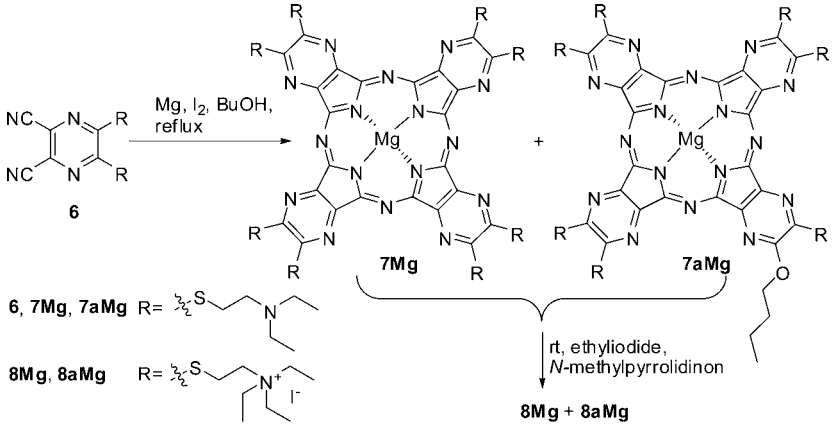


Figure 1. Structures of investigated compounds with the compound numbering.

Alkylsulfanyl and alkylamino AzaPcs are generally stable under Linstead cyclotetramerization using magnesium butoxide,[2,26] although some exceptions may be found.[27] These conditions were also applied for the synthesis of **7Mg** (Scheme 1); however, elemental analysis, NMR, and mass spectra indicated an exchange of some peripheral substituents for the butoxy ones (Supporting Information, Figures S1 and S2), which led to the compound **7aMg** (Scheme 1). Using a molar ratio of 30:1 for magnesium butoxide:**6**, the molar ratio of the products **7Mg**:**7aMg** was 5:1. Unexpectedly, lower molar excesses of magnesium butoxide, i.e., 20:1, 10:1, 7:1, and 4:1, led to products with a higher number of the butoxy substituents on the AzaPc macrocycle. In addition to the mass and NMR spectra, the exchange can be monitored using UV−vis spectroscopy because the presence of the butoxy substituents is indicated by a blue shift of the Q-bands (Supporting Information, Figure S3). The explanation of the reverse magnesium butoxide concentration dependency can be deduced from a reaction mechanism formulated recently for the transetherification of aryloxy-substituted AzaPc.[28] The exchange reaction proceeds on the starting compounds (pyrazine-2,3-dicarbonitriles) before the cyclotetramerization reaction is completed. Thus, at a high excess (e.g., 30:1 ratio), compound **6** cyclotetramerizes to **7Mg** more rapidly than the substituents are exchanged, and the formation of **7aMg** is minimized. Because the compounds **7Mg** and **7aMg** have the same R_f values in all of the solvents used, the mixture of **7Mg** and **7aMg** (molar ratio 5:1) was subsequently quaternized with ethyl iodide to a water-soluble mixture of **8Mg** and **8aMg** (Scheme 1). All attempts to avoid the exchange reaction using the template effect by heating **6** with magnesium acetate or magnesium chloride in quinoline, pyridine, or DMF led to dark

Scheme 1. Synthesis of Compound 8



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decomposition products, probably because of a low stability of **6**.[16]

The exchange of peripheral substituents for alkoxides complicates the synthesis of aryloxy-substituted AzaPcs using the Linstead method.[26,29] It has represented a problem for a long time, and the first syntheses of aryloxy-substituted AzaPcs have only been reported recently.[13,17,30] The successful synthesis method was based on the cooperative template effect of zinc(II), cobalt(II), or nickel(II) in high-boiling solvents and was adopted for the synthesis of **2Mg**. Thus, the heating of the 5,6-bis(2,6-diisopropylphenoxy)pyrazine-2,3-dicarbonitrile in anhydrous quinoline with anhydrous magnesium acetate under inert atmosphere provided **2Mg** in 42% yield. The importance of the template effect of magnesium(II) cations on the formation of the AzaPc macrocycle can be demonstrated by the fact that the same reaction in the absence of a magnesium(II) salt proceeded very slowly and yielded dark-brown undefined products. The only AzaPc in the reaction mixture was **2H** with a low yield of 4%.

Complexation of Magnesium(II) by AzaPc and Pc. Despite a number of reports of the complexation of metal cations (e.g., zinc(II), nickel(II), copper(II)) by metal-free Pc or AzaPc having been published, methods for the insertion of magnesium(II) have rarely been described. The procedures published thus far are not suitable for effective syntheses because they focus on a kinetic studies without isolation of the products,[31] use severe conditions supported by microwave irradiation,[32] or lead only to moderate yields.[33] Therefore, the Linstead method that uses magnesium alkoxides[33,34] or the method based on the cooperative template effect of magnesium(II) cations[35] are often employed to obtain the corresponding magnesium(II) derivatives. However, a problem arises in that magnesium(II) derivatives are often difficult to purify because of extensive tailing on silica columns (Supporting Information, Figure S4) in contrast to metal-free derivatives. Tailing of magnesium(II) complexes also precludes the isolation of unsymmetrical congeners obtained by statistical condensation, as recently reported.[36] Therefore, if an unsymmetrical magnesium(II) derivative is supposed to be a final product, the mixture of magnesium(II) congeners must be converted to the corresponding metal-free macrocycles, purified, and finally metalated with magnesium(II). This procedure, however, introduces problems with efficient magnesium(II) insertion. In the following section, we have optimized, for the first time, the conditions for the insertion of magnesium(II) with a high efficiency.

The pyrrole NH of metal-free AzaPcs and Pcs are weakly acidic, with pK_a values of 5.82 and 11.23, respectively, in DMSO.[37] These pK_a values are lower than those for structurally similar porphyrazines, which exhibit a pK_a of 12.36 (DMSO).[37] The complexation of magnesium(II) by porphyrazines[38] has been widely investigated, and the results indicate that the same reaction will proceed comparably or even better with Pcs and AzaPcs that are more acidic. As presented below, we have considered several factors that can be decisive for the reaction, including the magnesium(II) source, the solvent, the use of anhydrous conditions, the temperature, the type of macrocycle used, and the peripheral substitution. The reaction was optimized using **1H**, and the best conditions were used for **2H**−**5H** (Table 1).

The effects of several magnesium(II) salts (acetate, chloride, lactate, and carbonate) and MgO on **1H** metalation were compared. In anhydrous pyridine under reflux, the conversion

Table 1. Reaction Times and Yields of the Conversion of the Metal-Free Pcs and AzaPcs to Magnesium(II) Complexes

compound	core	connecting heteroatom	full conversion to Mg(II) complex (h)[a,c]	yield (%)
1	AzaPc	S	0.5	99
2	AzaPc	O	0.5	99
3	AzaPc	N	36[b]	93
4	Pc	S	0.5	96
5	Pc	O	10	99

[a]Monitored on TLC. [b]Traces of **3H** still detected. [c]Anhydrous $Mg(CH_3COO)_2$ (10 equiv), anhydrous pyridine, reflux.

to **1Mg** was completed after 30 min when magnesium acetate was used. The reaction slowed considerably with the use of magnesium chloride (6 h), whereas a barely detectable amount of **1Mg** was found after 6 h with other salts or MgO. Subsequently, the metalation with magnesium acetate was tested in several anhydrous solvents: pyridine, DMF, DMSO, *N*,*N*-dimethylacetamide, and THF. The full conversion was detected after 30 min in all of the investigated solvents except for THF, where only traces of the product were observed after 6 h. Interestingly, the results are consistent with the fact that all successful solvents form a proton-transfer complex with **1H**, as confirmed using UV−vis spectroscopy (Figure 2 and Figure S5

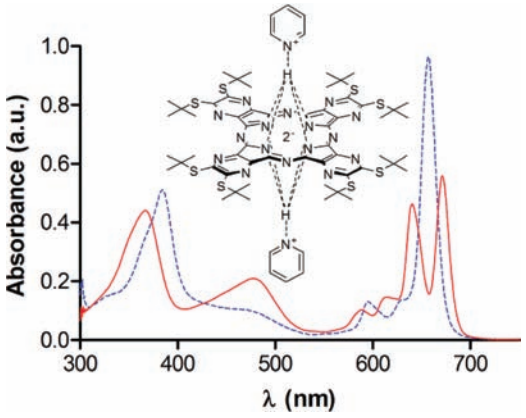

Figure 2. Absorption spectra of about 4.5 μM **1H** in THF (full line) and pyridine (dashed line): (inset) schematic structure of the proton-transfer complex of **1H** with pyridine.

in the Supporting Information).[39] The metal-free **1H** is unsymmetrical due to the presence of two central hydrogen atoms (D_{2h} symmetry); as a consequence, the absorption Q-band splits into two bands in noninteracting solvents such as THF (Figure 2). However, the formation of the proton-transfer complexes of Pcs and AzaPcs is accompanied by an increase in the molecular symmetry (D_{4h} symmetry), as indicated by the observation of only one intense Q-band. This band is observed in all of the interacting solvents (Figure 2).[15] Possible formation of the pure dianionic form of metal-free macrocycle in these solvents was excluded based on the different absorption spectra as compared to those obtained previously.[15]

The importance of the formation of the proton-transfer complex for the metalation to complete was proved in THF with increasing amounts of pyridine (2, 10, 100, or 1000 equiv of **1H**). When 1000 equiv were used, the metalation was completed in 30 min. No reaction occurred with 2 or 10 equiv after 6 h, whereas the presence of 100 equiv led to approximately 10% conversion. The binding of the solvent

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Table 2. Photophysical and Photochemical Data for the Investigated Compounds in THF at Room Temperature[a]

compound	core	A	λ_{max}/nm	λ_F/nm	$\Delta\lambda$/ cm^{-1}	Φ_Δ[b]	Φ_F[b]	τ_F/ns	k_r/s^{-1} × 10^{-8}	k_{nr}/s^{-1} × 10^{-8}	Φ_{Ph}[b,c] ×10^5
1Zn	AzaPc	S	649	656	164	0.55	0.35	2.66	1.3	2.4	0.17
1Mg	AzaPc	S	651	658	163	0.30	0.53	5.45	1.0	0.9	0.20
1H	AzaPc	S	640, 670	675	111	0.09	0.08	0.9 (90%)	0.9[e]	10.2[e]	
								~2 (10%)			
2Zn	AzaPc	O	624	630	153	0.50	0.41	2.78	1.5	2.1	0.74
2Mg	AzaPc	O	626	633	177	0.31	0.73	5.50	1.3	0.5	0.94
2H	AzaPc	O	608, 646	651	119	0.11	0.17	1.78	1.0	4.7	
3Zn	AzaPc	N	653	663	231	0.02	~0.001				0.014
3Mg	AzaPc	N	656	662	138	0.01	~0.002	0.73			0.0058
3H	AzaPc	N	648, 679	n.d.[c]	n.d.[c]	0.001	n.d.[d]				
4Zn	Pc	S	697	707	203	0.68	0.16	2.76	0.6	3.0	1.05
4Mg	Pc	S	699	709	202	0.23	0.23	6.03	0.4	1.3	1.73
4H	Pc	S	694, 722	732	189	0.23	0.19	5.77	0.3	1.4	
5Zn	Pc	O	675	680	109	0.61	0.29	3.65	0.8	2.0	2.40
5Mg	Pc	O	677	681	87	0.26	0.52	7.34	0.7	0.7	2.40
5H	Pc	O	663, 701	704	61	0.28	0.33	6.71	0.5	1.0	
ZnPc	Pc		666	671	112	0.53[h]	0.32	3.38	0.9	2.0	
ZnPc[c]	Pc		669	674	111		0.28	3.30	0.8	2.2	2.23[j]
ZnPc[f]	Pc		673	679	131		0.28	3.39	0.8	2.1	
ZnPc[g]	Pc		678	684	129		0.30[i]	3.53	0.8	2.0	

[a]The connecting heteroatom between the macrocycle core and substituent (A), the absorption Q-band maximum (λ_{max}), the fluorescence band maximum (λ_F), the Stokes shift ($\Delta\lambda$), the quantum yield of singlet oxygen formation (Φ_Δ), the quantum yield of fluorescence (Φ_F), the lifetime of the excited singlet states (τ_F), the radiative (k_r) and nonradiative (k_{nr}) rate constants, and the quantum yield of photodecomposition (Φ_{Ph}). [b]Mean of three measurements; estimated error, ±15%. [c]Measured in air-saturated DMF; metal-free compounds were not measured because of the different extent of the proton-transfer complex formation. [d]Not detected. [e]Rate constants for the major process. [f]In pyridine. [g]In 1-chloronaphthalene. [h]Reference 20. [i]Reference 21. [j]Reference 22.

evidently weakens the central pyrrole N–H bond and facilitates the insertion of magnesium(II) cations into the macrocycle.[40]

In general, temperature influences reaction rates. In our case, the full conversion of **1H** to **1Mg** is accomplished in 30 min in pyridine with magnesium acetate at 90 °C or under reflux, whereas a reaction time of 3 h was required at 70 °C, and no metalation occurred at 45 °C or at room temperature after 48 h. Anhydrous conditions do not appear to be necessary for successful metalation. The use of anhydrous magnesium acetate or the corresponding tetrahydrate, anhydrous pyridine, or the commercially available pyridine (p.a. purity, 0.1% water) led to the same results.

The optimized conditions were used for metalation of **2H**–**5H** to determine whether the metalation conditions are suitable for both the AzaPc and Pc macrocycles bearing different substituents (Table 1). The reaction proceeded quantitatively, except for that of **3H**. The low reactivity of this compound can be attributed to the low acidity of the pyrrole NH groups due to a strong donating effect of the peripheral *N*,*N*-diethylamino substituents, which limits the formation of the proton-transfer complex. These results are consistent with previously published data that showed a very slow formation of the proton-transfer complex of **3H** and the similarly substituted AzaPcs[15,41] and emphasized the importance of this intermediate in the reaction mechanism.

Absorption Spectra. The investigated compounds exhibit absorption spectra typical of Pc and AzaPc, i.e., a high-energy B-band located between 350 and 390 nm and low-energy Q-band located at approximately 620–720 nm (Table 2, Figure 3, Figure S6 in the Supporting Information). Whereas the location and shape of the B-bands are only slightly sensitive to structural variations or aggregation effects, the Q-bands are considerably affected. Thus, the Q-bands split in the metal-free derivatives

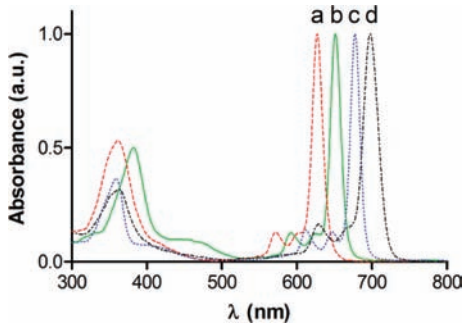

Figure 3. Absorption spectra of **1Mg** (b, full line), **2Mg** (a, dashed line), **5Mg** (c, dotted line), and **4Mg** (d, dashed dotted line) in THF. The spectra were normalized to the same absorption in the Q-band.

with D_{2h} symmetry (Figure 2) and are broadened upon aggregation. The aggregation was observed only for the compound **8Mg** in water; the absorption spectra of the other studied compounds (Figure 3, Figure S6 in the Supporting Information) indicated only monomeric species.

The positions of the Q-band maxima are influenced by several factors (Figure 3, Table 2). (i) Macrocycle core: The Q-bands of Pcs are always red-shifted by approximately 50 nm compared to the corresponding bands of AzaPcs. The observed shift supports recent theoretical calculations that state that nitrogen atoms in the pyrazine rings of AzaPcs stabilize the highest occupied molecular orbital and destabilize the lowest unoccupied molecular orbital.[42] (ii) Peripheral substituents: A large shift is caused by connecting heteroatoms. The aryloxy derivatives exhibit Q-bands blue-shifted by approximately 30 nm compared to the corresponding bands of the alkylsulfanyl or dialkylamino derivatives. (iii) Central metal: The magnesium(II) derivatives exhibit Q-bands red-shifted by

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approximately 2 nm compared to the bands of the zinc(II) derivatives.

Fluorescence and Singlet Oxygen Formation. The fluorescence emission spectra of the compounds mirrored the absorption spectra in the Q-band region (Figure 4). The Stokes

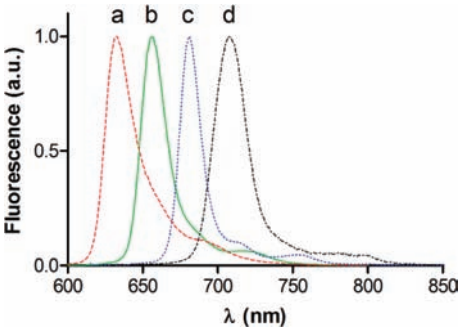

Figure 4. Normalized fluorescence emission spectra of **1Mg** (b, full line), **2Mg** (a, dashed line), **5Mg** (c, dotted line), and **4Mg** (d, dashed dotted line) in THF. The samples were excited in the Q-band region.

shifts were in the range 60−200 cm^{-1}, which is typical for Pc and AzaPc derivatives.[13,36] The fluorescence excitation spectra overlapped the corresponding absorption spectra, which confirmed the identity of the compounds (Figure S6 in the Supporting Information). The overlap also documents the only presence of the monomeric species (except for the compound **8Mg**, which partially aggregated in water) because the aggregates differ in absorption spectra, and most do not exhibit fluorescence.

The fluorescence quantum yields, Φ_F, were determined in THF using zinc(II) phthalocyanine (ZnPc) as a reference (Φ_F = 0.30 in 1-chloronaphthalene)[21] (Table 2). The Φ_F values are significantly higher for the magnesium(II) derivatives ($\Phi_F \sim$ 0.5−0.7) than for the zinc(II) and metal-free derivatives and are among the highest values reported for porphyrinoid compounds thus far (Table 3). Interestingly, AzaPcs have higher Φ_F

Table 3. Fluorescence Quantum Yields of Magnesium(II) Porphyrinoid Compounds

compound	Φ_F
2Mg	0.73 (THF)
5Mg	0.52 (THF)
chlorophyll a	0.35 (propanol),[43] 0.32 (ether)[44]
chlorophyll b	0.12 (benzene)[45]
magnesium(II) tetrabenzoporphyrin	0.50 (toluene)[43]
magnesium(II) tetraphenylporphyrin	0.13 (benzene)[21]
magnesium(II) octaphenylporphyrazine	0.23 (THF)[46]

values than Pcs with the same central metal and peripheral substitution. Thus, the highest Φ_F value was observed for **2Mg** (Φ_F = 0.73), whereas the corresponding derivative with the Pc core, **5Mg**, exhibited a Φ_F of 0.52. With respect to the effects of the peripheral substituents, the Φ_F values of AzaPc and Pc derivatives can be arranged as follows: aryloxy > alkylsulfanyl ≫ *N*,*N*-diethylamino substituents. The high Φ_F values for aryloxy derivatives are in good agreement with data presented recently for similar AzaPc with peripheral ether linkages.[28] The absence of significant fluorescence for the derivatives **3** indicates that excited-singlet-state relaxation is dominated by a competitive nonradiative pathway. The relaxation involves ultrafast intramolecular charge transfer (ICT) between the peripheral *N*,*N*-diethylamino substituents and the macrocycle core and decreases the quantum yields of both fluorescence and singlet oxygen (see below).[36]

The fluorescence emission properties were complemented by the fluorescence lifetimes (Table 2). The fluorescence lifetimes of the magnesium(II) derivatives are longer than those of the corresponding zinc(II) and metal-free derivatives (except for compounds **3** with ICT). The radiative, k_r, and nonradiative, k_{nr}, rate constants calculated from the experimental data according to $k_r = \Phi_F/\tau_F$ and $k_{nr} = (1 - \Phi_F)/\tau_F$, respectively, indicate the increased role of nonradiative processes in the relaxation of the zinc(II) and metal-free derivatives (Table 2). In the case of the zinc(II) derivatives, high Φ_Δ values suggest enhanced intersystem crossing. The metal-free derivatives have an increased internal conversion channel because the sum of Φ_F and Φ_Δ is much less than 1, which can be attributed to the enhanced rotational and vibrational motions of the macrocycle.

Absorption in the red region, high Φ_F, high photostability (see below), and simple syntheses of alkylsulfanyl magnesium(II) AzaPcs make these derivatives promising fluorescence probes for the visualization of biomembranes and the labeling of cells. Their function in a biological environment was tested by incorporation of **1Mg** into liposomes, which represent simple models of biomembranes.[47] As deduced from the absorption and fluorescence spectra, **1Mg** integrates into the lipid bilayers in the monomeric form (see Figure S6 in Supporting Information) and conserves its fluorescence properties (Φ_F = 0.64 in liposomes). To obtain soluble AzaPc in water, the octacationic compound **8Mg** was synthesized. The obtained apparent Φ_F value of 0.25 is affected by partial aggregation in water; however, this value is still high compared with those of other porphyrinoid compounds (Table 3).

The quantum yields of singlet oxygen formation,Φ_Δ, are summarized in Table 2. The zinc(II) complexes are evidently better producers of $O_2(^1\Delta_g)$ than are the corresponding magnesium(II) complexes. Furthermore, derivatives with Pc cores are more effective than those with the AzaPc core, and the presence of the alkylsulfanyl substituents leads to higher Φ_Δ than do the aryloxy substituents. The compounds **3** have negligible Φ_Δ that is caused by competitive ICT.[36]

Photochemical Stability. High photostability is one of the parameters that determine the suitability of a compound as a fluorescence probe. Photoproduced $O_2(^1\Delta_g)$ is believed to be a decisive species that is responsible for the photodecomposition of Pcs and AzaPcs.[48,49] Photostability, as quantified by the quantum yields of photodecomposition, Φ_{Ph}, was measured by absorption spectroscopy during the continuous irradiation of DMF solutions. The measurements showed the gradual disappearance of all absorption bands that is typical for a destruction of the macrocycle (Supporting Information, Figure S7). The Φ_{Ph} values summarized in Table 2 show that the AzaPc derivatives are several times more stable than the corresponding Pcs. The lowest photostability was observed for the Pcs derivatives **5Zn** and **5Mg** and was also indicated by their decomposition in daylight during the synthesis and purification steps. On the other hand, **1Mg** and **1Zn** exhibited the best photostability, which suggests that alkylsulfanyl AzaPcs derivatives are potential fluorescent probes. Extremely low photodecomposition of compounds **3** results from the very low production of $O_2(^1\Delta_g)$. The presented results are consistent with the findings that tetrapyrazinoporphyrazines are among

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the most photostable in the group of Pcs and their azanalogues.[48]

Photophysical Properties and Consequences for Applications. According to the presented results, the magnesium(II) AzaPcs are prospective red-emitting fluorescent probes. Focusing on the peripheral substitution, the alkylsulfanyl derivatives appear to be the compounds of a choice for several reasons: (i) The compound **1Mg** exhibits a slightly lower Φ_F than the corresponding aryloxy **2Mg** derivative; however, **1Mg** exhibits an absorption in the Q-band region approximately two times greater than that of **2Mg** (approximately 3×10^5 and 1.84×10^5 M^{-1} cm^{-1} for **1Mg** and **2Mg**, respectively). The intensive absorption in the excitation area is advantageous because it increases the brightness of the emitted signal. (ii) A positive feature of the alkylsulfanyl derivative **1Mg** is its high photostability. Extensive photobleaching of the fluorophores limits their use in fluorescence microscopy, particularly when longer irradiation times are applied. (iii) The synthetic methods for the alkylsulfanyl derivatives are simpler because of their higher stability against exchange of the peripheral substituents compared to the corresponding aryloxy AzaPcs.[26] (iv) Another important factor is that alkylsulfanyl AzaPcs absorb and emit in the area that overlaps the widely used red cyanine Cy5 fluorophore (Figure 5) that is considered

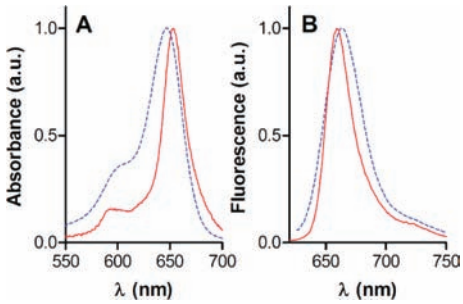

Figure 5. Absorption (A) and emission (B) spectra of **8Mg** in water (full line) and of Cy5™ in phosphate buffer (dashed line). The excitation wavelength was 610 nm.

as a fluorescence standard for the red portion of the spectrum. A number of selective optical filter sets have been designed for Cy5 and are widely used in fluorescence microscopy (e.g., Cy5HYQ from Nikon and U-DM-Cy5 from Olympus). Both the absorption and emission spectra of alkylsulfanyl AzaPc fit perfectly with these filter sets. In contrast, the aryloxy AzaPcs, in general, suffer from a 30 nm blue shift that places the absorption and emission spectra in a nonoptimal spectral overlap. The optical and photophysical properties of **1Mg** are superior to those of Cy5. Cy5 exhibits a lower Φ_F (0.40 in ethanol)[50] and a lower molar absorption coefficient (2.50×10^5 M^{-1} cm^{-1} at 658 nm in ethanol) and suffers from low photostability. Moreover, in contrast to Cy5, which absorbs significantly only in the red spectral region, AzaPcs, in general, benefit from another strong absorption band at approximately 380 nm (B-band) that extends up to 500 nm (Figure 3). The B-band allows a broader choice of the excitation wavelengths. (v) AzaPcs can be incorporated into biomembranes without the loss of fluorescence, as confirmed for **1Mg** anchored into liposomes. (vi) The alkylsulfanyl derivatives can be further modified to obtain water-soluble fluorescent derivatives (e.g., **8Mg**). Despite partial aggregation of this compound in water, the derivative **8Mg** exhibits a Φ_F of 0.25, which is comparable to that of Cy5 (Φ_F = 0.27 in PBS[50]).

CONCLUSION

The syntheses of magnesium(II) Pcs and AzaPcs have been achieved. The optimized conditions for the complete conversion of the metal-free compounds involve reflux in pyridine in the presence of magnesium acetate. Under these conditions, the derivatives with strong electron-donating substituents are also metalated. Photophysical and photochemical studies indicate high fluorescence quantum yields of the magnesium(II) AzaPcs compared to those of the corresponding zinc(II) and metal-free derivatives. The results demonstrate that the AzaPc core is well-suited to the construction of novel efficient fluorophores. In particular, the alkylsulfanyl AzaPcs exhibit Φ_F values greater than 0.50, absorption molar coefficients of approximately 3×10^5 M^{-1} cm^{-1}, and considerable photostability, which makes them potential fluorescence probes for use in organic solvents, biomembranes, or aqueous media. We envisage that the alkylsulfanyl AzaPcs will find applications as excellent red fluorophores with properties superior to those of commercially used Cy5.

ASSOCIATED CONTENT

Supporting Information

New analytical data for known compounds, experimental procedures for quantum-yield determinations, ^{1}H NMR spectra, and additional absorption and fluorescence spectra. This information is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Tel.: +420 495067257. Fax: +420 495067167. E-mail: petr.zimcik@faf.cuni.cz.

Notes

The authors declare no competing financial interest.

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