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Photophysical properties and photochemistry of a sulfanyl porphyrazine bearing isophthaloxybutyl substituents

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ABSTRACT

A magnesium porphyrazine possessing isophthaloxybutylsulfanyl substituents in the periphery was synthesized and subjected to various photophysical studies, including optical absorption and emission measurements. Moreover, synchronous fluorescence spectra were recorded and a contour three-dimensional map of the excitation-emission of the studied porphyrazine was obtained. The porphyrazine macrocycle exhibited interesting solvatochromic effects in many different solvents. Upon excitation with visible light, it generated singlet oxygen with a low quantum yield, therefore when it was encapsulated in liposomes it exhibited no photocytotoxicity in the *in vitro* study on human carcinoma LNCaP cell line.

1. Introduction

Porphyrazines (Pzs) are synthetic analogues of naturally occurring porphyrins. The Pz macrocycle consists of four pyrrole rings linked together with azamethine groups [1]. Pzs show unique spectrochemical properties and potential applications in technology and medicine, especially as photosensitizers in photodynamic therapy (PDT) [2–5]. Pzs with peripheral sulfanyl substituents revealed enhanced solubilities and higher singlet oxygen generation yields [6–9]. The main limiting factors for Pzs application in PDT are their poor solubility in water, tendency to form aggregates and photochemical instability. These drawbacks may be overcome by modifying Pzs periphery [1,10] or by their encapsulation in various drug delivery systems, of which liposomal formulations and dendrimeric architectures have shown potential for other azaporphyrins [11,12].

In this study we report the synthesis and photochemical characterization of a novel sulfanyl magnesium(II) porphyrazine with isophthaloxybutyl substituents.

2. Experimental section

2.1. Materials

2.1.1. 2,3-Bis[4-(3,5-dimethoxycarbonylphenoxy)butylsulfanyl] maleonitrile (*3*)

Dimercaptomaleonitrile disodium salt (465 mg, 2.50 mmol) and dimethyl 5-(4-bromobutoxy)isophthalate **2** (2.15 g, 6.25 mmol) were dissolved in anhydrous methanol (50 mL). The reaction mixture was stirred under reflux for 6 h. The solvent was evaporated and the residual brown oil was chromatographed (dichloromethane: methanol, 50:1, v/v) to give **3** as yellow-brown oil (0.91 g;

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54% yield), which when refrigerated tends to solidify to yellow amorphous solid. M.p. = 75–81 °C; R_f (dichloromethane: methanol, 50: 1, v/v) 0.56; UV–Vis (dichloromethane) λ_{max} nm (log ε) 318 (4.13), 343 (4.23); ^{1}H NMR (500 MHz, pyridine-d_5) δ 8.52 (s, 2H, C4′, ArH), 7.91 (s, 4H, C2′, C6′, ArH), 3.97 (t, ^3J = 6.0 Hz, 2H, $SCH_2CH_2CH_2\underline{CH_2}$), 3.87 (s, 12H, $COOCH_3$), 3.31 (t, ^3J = 6.5 Hz, 4H, SCH_2), 1.92 (m, 4H, $SCH_2CH_2\underline{CH_2}$), 1.91 (m, 4H, $SCH_2\underline{CH_2}CH_2$); ^{13}C NMR (125 MHz, pyridine-d_5) δ 166.5 (C = 0), 159.9 (CH_2–O–C, ArC), 132.8 (C–CO, ArC), 123.5, (ArC), 122.3 (CN), 120.5 (ArC), 113.5 (NC–C–S), 68.3 (O–CH_2, Bu), 52.8 ($COO\underline{CH_3}$), 35.4 (S–CH_2, Bu), 28.5 ($SCH_2\underline{CH_2}$, Bu), 27.4 ($SCH_2CH_2\underline{CH_2}$, Bu); MS (ES pos) *m/z* 693 $[M+Na]^+$, 709 $[M+K]^+$. MS (ES neg) *m/z* 705 $[M+Cl]^-$. Anal. Calc. for $C_{32}H_{34}N_2O_{10}S_2$: C, 57.30; H, 5.11; N, 4.18; S, 9.56. Found: C, 57.46; H, 5.62; N, 4.20, S, 9.54.

2.1.2. 2,3,7,8,12,13,17,18–Octakis[4-(3,5-dibutoxycarbonylphenoxy) butylthio]porphyrazinato magnesium(II) (**4**)

Magnesium turnings (11 mg, 0.45 mmol) and a small crystal of iodine were refluxed in *n*-butanol (10 mL) for 4 h. After cooling to room temperature, the reaction mixture was transferred using a syringe to a flask containing maleonitrile **3** (233 mg, 0.34 mmol), and was heated under reflux for 22 h. Next, the reaction mixture was cooled to room temperature, filtered through Celite, which was then washed with toluene. Solvents were evaporated in a rotary evaporator, which resulted in a dark blue residue, and was chromatographed using silica gel (dichloromethane: methanol, 50: 1, v/v) and reverse phase column chromatography (methanol, than dichloromethane) to give **4** as dark blue film (111 mg; 37% yield). R_f (*n*-hexane: ethyl acetate, 7: 3, v/v) 0.44; UV–Vis (dichloromethane) λ_{max} nm (log ε) 317 (4.77), 378 (4.89), 501 (4.17), 611 (4.45), 672 (4.94); ^{1}H NMR (500 MHz, pyridine-d_5) δ 8.50 (s, 8H, C4′, ArH), 7.87 (s, 16H, C2′, ArH), 4.59 (s, 16H, SCH_2), 4.34 (t, ^3J = 6.5 Hz, 32H, $COOCH_2$), 4.15 (s, 16H, $SCH_2CH_2CH_2\underline{CH_2}$), 2.35 (bs, 32H, $SCH_2\underline{CH_2}\underline{CH_2}$), 1.64 (m, 32H, $COOCH_2\underline{CH_2}$), 1.35 (m, 32H, $COOCH_2CH_2\underline{CH_2}$), 0.87 (t, ^3J = 7.5 Hz, 48H, $COOCH_2CH_2CH_2\underline{CH_3}$); ^{13}C NMR (125 MHz, pyridine-d_5) δ 166.0 (C = 0), 160.0 (CH_2–O–C, ArC), 158.3 (N=C Ar), 141.6 (C–S, Ar), 133.0 (C–CO, ArC), 123.2 (ArC), 120.2 (ArC), 68.8 (ArO-$\underline{CH_2}$), 65.8 ($COO\underline{CH_2}$), 35.7 (S–CH_2), 31.4, 29.3, 28.0, 19.9, 14.3 (CH_3); MS (MALDI) *m/z* 3378 $[M+H]^+$. HRMS (ESI) Calc. for $C_{176}H_{233}MgN_8O_{40}S_8$: 3378.4060, Found: $[M+H]^+$ 3378.4009. HPLC purity 96.22–100.00% (Supplementary Content).

2.2. UV/Vis measurements

All solutions containing **4** were prepared prior to their absorbance, steady-state fluorescence, and fluorescence excitation measurements. UV–Vis absorption spectra were recorded on a JASCO V-650 spectrophotometer in the spectral range from 300 nm to 800 nm, whereas the emission spectra (steady-state fluorescence excitation and emission spectra, synchronous fluorescence spectra and 3D fluorescence spectra) were recorded on a Jobin Yvon-Spex Fluorolog 3-22 spectrofluorometer. Fluorescence quantum yields were calculated using quinine sulphate in 0.05 M H_2SO_4 as a reference for $S_2 \rightarrow S_0$ emission (Φ_F^{st} = 0.546) [13] and using zinc phthalocyanine (ZnPc) in DMF (Φ_F^{st} = 0.17) [14] for $S_1 \rightarrow S_0$ emission. Fluorescence quantum yields were calculated according to the equation below:

$$\Phi_F = \Phi_F^{st} \frac{\int F_X\left(1-10^{-A_{st}}\right)}{\int F_{st}\left(1-10^{-A_X}\right)} \frac{(n_X)^2}{(n_{st})^2} F_k \qquad (1)$$

here, $\int F_x$ is the area under the emission curve of the sample, $\int F_{st}$ is the area under the emission curve of the standard, and A_x and A_{st} are the absorbance of the sample and standard at an excitation wavelength, respectively, n_x – the solvent refractive index for the sample, n_{st} – the solvent refractive index for the standard, F_k – the constant describing the instrumental factors, including geometry and other parameters, Φ_F^{st} is the value of fluorescence quantum yield of the standard.

Synchronous fluorescence spectra (SFS) were collected by simultaneous scanning using the excitation and emission monochromators, in the range from 290 nm to 750 nm at $\Delta\lambda$ = 10, 20, 30, 40, 60, 80, 100, and 120 nm. However, after the preliminary selection only the data collected for $\Delta\lambda$ = 20 nm were discussed below. A contour map of the emission-excitation of **4** was obtained in acetonitrile by recording the emission spectra in the range from 350 nm to 750 nm using the excitation wavelengths from 300 nm to 400 nm, spaced by 5 nm intervals in the excitation domain.

Fluorescence lifetime measurements were made at the Centre for Ultrafast Laser Spectroscopy in Poznan, with the respective fluorescence lifetime spectrophotometer setup. Time-Correlated Single Photon Counting (TCSPC) technique, previously described in detail elsewhere [15], was applied. Spectra-Physics pico/femtosecond laser system was used as the source of exciting pulses. This included a Tsunami Ti: sapphire laser, pumped with a BeamLok 2060 argon ion laser, which generated 1–2 ps pulses at a repetition rate of about 82 MHz and average power of over 1 W, tunable in the 720–1000 nm range. The repetition rate of the excitation pulses varied from 4 MHz to a single-shot by using a model 3980-2S pulse selector. Second and third harmonics of the picosecond pulse obtained on a GWU-23PS harmonic generator could be used for excitation, giving greater flexibility in the choice of the excitation wavelength. Elements of an Edinburgh Instruments FL900 system were used in the optical and control components of the system. The pulse timing and data processing systems employed a biased TAC model TC 864 (Tenelec) and a R3809U-05 MCP-PMT emission detector with thermoelectric cooling and appropriate preamplifiers (Hamamatsu).

2.3. Singlet oxygen generation study

A singlet oxygen generation assay was performed according to the procedure described in detail by Sobotta et al. [16]. Irradiation was performed at 671 nm according to the Q-band maximum wavelength of **4** in DMF.

Further we used a Jobin Yvon-Spex Fluorolog 3-22 spectrofluorometer with H10,330B-75 NIR-PMT module to determine the values of quantum yield of singlet oxygen generation of **4**. Macrocycle was excited at 380 nm in acetonitrile in order to record luminescence of singlet oxygen at 1270 nm.

2.4. Liposome preparation

1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and L-α-phosphatidyl-DL glycerol (chicken egg, PG) were purchased from Avanti Polar Lipids– INstruchemie (Delfizijl, Netherlands). Liposomes with **4** were prepared by a thin-film hydration method [17,18]. Appropriate amounts of the lipid solutions in chloroform (25 mg/mL) and **4** (0.8 mg/mL) were placed in glass test tubes, mixed and evaporated to dryness using a rotary evaporator. Films formed on the bottom of the glass test tubes were dried overnight in a vacuum at room temperature to evaporate any remaining chloroform. Subsequently, the dried films were hydrated with HEPES buffered saline solution (10 mM HEPES, *N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid), 140 mM NaCl, pH = 7.4) and dispersed by vortexing for 10 min using a Vortex Genie 2 digital. Resulting liposome suspensions were passed 21 times through polycarbonate membranes with a pore diameter of 100 nm, using a