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Chemical Physics Letters 418 (2006) 355-358



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# Photophysical properties of gallium hydroxyl tetratolylporphyrin and $13^2$ -demethoxycarbonyl-(gallium hydroxyl)–methyl-pheophorbide *a*

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Received 11 July 2005; in final form 27 October 2005 Available online 28 November 2005

#### Abstract

Two metal tetrapyrroles containing gallium, gallium hydroxyl tetratolylporphyrin and  $13^2$ -demethoxycarbonyl-(gallium hydroxyl)– methyl pheophorbide *a* (Ga-(OH)-chlorin), were synthesized from their respective free bases using Ga(III)-acetylacetonate in a phenol melt. Their photophysical properties were investigated and the quantum yields of different monomolecular deactivation processes were determined. For Ga-(OH)-porphyrin S<sub>2</sub>-fluorescence was observed and quantified. In contrast, for Ga-(OH)-chlorin no S<sub>2</sub>-fluorescence was observed. Both compounds should be useful as efficient photosensitizers in photodynamic therapy. © 2005 Elsevier B.V. All rights reserved.

#### 1. Introduction

Metal porphyrin derivatives are biologically important chromophores with a macrocyclic-conjugated system of  $\pi$ -electrons. The synthesis and photophysical characterization of metal porphyrins and especially the diamagnetic Ga-porphyrin-complexes were and still are a hot topic in chemistry and optical spectroscopy [1–4]. Characteristic and partly intense B and Q absorption bands in the nearultraviolet and visible regions, respectively, arise from the  $(\pi \rightarrow \pi^*)$  transitions of the electronic system of the porphyrin. In case of closed shell configuration of the central metal, the outer-shell electrons give only a minor perturbation [3,5].

Specifically, several reports on Ga-porphyrin-complexes have appeared in the past, where both synthetic procedures and photophysical studies were in the center of interest [1– 4]. Insertion of gallium into porphyrins has been achieved by two different synthetic approaches in the past: reaction of the free base with gallium (III)-salts, e.g. chloride, in acetic acid [6] and by the solvent-free procedure by reacting the porphyrin with gallium (III)-acetylacetonate in a phenol melt [7].

Interestingly, no photophysical studies on gallium chlorins have been published to our knowledge, and preparative chemistry is largely unknown. Generally, synthetic procedures and photophysical properties of free base chlorins and of a large variety of metal chlorins, especially chlorophyll *a* derivatives, have been studied extensively for decades [8–15]. Related gallium containing species, however, do not seem to have gained much interest, even though the additionally available axial position in gallium centered tetrapyrroles could be particularly useful as a versatile coupling site for the synthesis of novel donor–acceptor systems. Such novel dyads could be utilized in the investigation of photoinduced energy or electron transfer.

Moreover, on the way towards 2nd and 3rd generation photosensitizers for photodynamic therapy (PDT) [16], besides a number of porphyrins, certain chlorins were considered as prospective photosensitizing molecules [17]. Their characteristic absorption spectra with bands in the far red spectral region with large extinction coefficients combined with high quantum yields for singlet oxygen generation [18] suggest their potential efficiency in photodynamic activity.

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In 1971, Bajema et al. [19] first observed S<sub>2</sub>-fluorescence of a porphyrin: zinc tetrabenzoporphyrin (zinc tetraphenylporphyrin) in octane and in argon matrices. Since that time emission from higher excited states was observed for a variety of diamagnetic metal porphyrins [3,5,19–24]. The large energy gap (Ga-(OH)-TTP: 6800 cm<sup>-1</sup>) and the parallel energy surfaces of S<sub>1</sub> and S<sub>2</sub> state retard the internal conversion between the two states. Therefore a greater transition moment granted to S<sub>2</sub>  $\rightarrow$  S<sub>0</sub> transition yields emission from the S<sub>2</sub> state [5]. Ohno et al. investigated the S<sub>2</sub>-fluorescence of different metal porphyrins and also for a gallium porphyrin (Ga-Cl-TPP in benzene) [3].

The steady state  $S_2$ -fluorescence can be used as a reference to determine the rates of photoinduced electron transfer (PET) in, e.g., thin layers like Langmuir–Blodgett-films, where the fluorescence intensity is very low and time resolved measurements become impossible. Due to the fact that only the  $S_1$  contributes to the PET, the ratio between the intensities of  $S_1$ - and  $S_2$ -fluorescence can be considered as measure of PET rates [25].

In a comparative study we investigated the photophysical properties of Ga-(OH)-TTP and a novel semi synthetic Ga-(OH)-chlorin derived from chlorophyll a, and evaluated their suitability as either electron donor in electron transfer systems or as efficient photosensitizer for PDT. This study is expected to re-focus attention on gallium containing tetrapyrroles and to stimulate further preparative-synthetic and photophysical investigations.

## 2. Materials and methods

# 2.1. Sample preparation and analysis

Tetratolylporphyrin, gallium acetylacetonate, phenol and common laboratory solvents were obtained from Sigma–Aldrich, Taufkirchen, Germany. 13<sup>2</sup>-Demethoxycarbonyl methylpheophorbide *a* was obtained by extracting and processing the blue–green algae *Spirulina maxima* following a procedure of Smith et al. [26]. NMR-spectra were recorded using a Bruker DRX-300 NMR spectrometer (300 MHz for <sup>1</sup>H). Mass analysis was performed with a Finnigan MAT 90 spectrometer and UV/VIS spectra were recorded on a Shimadzu 160A spectrometer.

Tetratolylporphyrin-hydroxy-gallium (III)-complex(1) was synthesized according to the literature procedure by Buchler and Puppe [7], starting from the TPP free base, phenol and gallium (III)-acetylacetonate. The NMR and mass spectroscopic data are in accordance with the literature reports of the first published synthesis of this compound by Eaton et al.[6].

 $13^2$ -Demethoxycarbonyl methylpheophorbide a-hydroxygallium (III)-complex(2). In modification of the above procedure, 300 mg (0.35 mmol)  $13^2$ -demethoxycarbonyl methylpheophorbide a, 125 mg (0.35 mmol) Ga(III)-acetylacetonate and 1 g phenol in a 10 ml flask under argon and heated for 15 min at 140 °C on a metal bath. Subsequently the excess phenol was removed by Kugelrohr distillation under reduced pressure. The crude reaction mixture was subjected to flash chromatography on silica to hydrolyze the phenoxide, using THF/MeOH (9:1) as eluent. The major blue–green band was collected and purified by chromatography on neutral aluminium oxide, eluting with chloroform to give 110 mg (35%) as a blue–green solid.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): 9.14 (s, 1H, 10-H), 9.01 (s, 1H, 5-H), 8.39 (s, 1H, 20-H), 7.88 (dd,  $J_{3-1,3-2A} = 17.7$  Hz,  $J_{3-1,3-2B} = 11.3$  Hz, 1H, 3<sup>1</sup>-H), 6.14 (dd,  $J_{3-2A,3-2B} = 1.7$  Hz,  $J_{3-2A,3-1} = 17.8$  Hz, 1H, 3<sup>2A</sup>-H), 6.04 (dd,  $J_{3-2B,3-2A} =$ 1.7 Hz,  $J_{3-2B,3-1} = 11.5$  Hz, 1H, 3<sup>2B</sup>-H), 4.82 (2d, J = 19.7 Hz, 2H, 13<sup>2</sup>-CH<sub>2</sub>), 4.40 (m,  $J_{17,18} = 1.9$  Hz,  $J_{18,18Me} = 7.1$  Hz, 1H, 18-H), 4.15 (m, 1H, 17-H), 3.48 (q, J = 7.6 Hz, 2H, 8<sup>2</sup>-CH<sub>2</sub>), 3.32 (s, 3H), 3.03 (t, J = 6.3 Hz, 2H, 17<sup>2</sup>-CH<sub>2</sub>), 3.31 (s, 3H), 3.22 (s, 3H), 2.52 (m, 1H, 17<sup>1A</sup>-H), 2.21 (m, 1H, 17<sup>1B</sup>-H), 1.83 (d, J = 7.2 Hz, 3H, 18-Me), 1.55 (t, J = 7.6 Hz, 3H, 8<sup>1</sup>-Me), 1.43 (m, 1H, 17<sup>2A</sup>-H), 1.25 (m, 1H, 17<sup>2B</sup>-H).

EI-MS: *m*/*e*: ber. für C<sub>34</sub>H<sub>35</sub>GaN<sub>4</sub>O<sub>4</sub>: 633,4, gef.: 633,2 (10%, M<sup>+</sup>), 616 (60%, M–OH).

#### 2.2. Steady state absorption and fluorescence

Ground state absorption spectra were recorded at room temperature using the commercial spectrophotometer Shimadzu UV160A. The emission spectra of the compounds dissolved in toluene were taken at room temperature in a 1 cm × 1 cm optical quartz cell using a Ti:Sapphire-laser (Mira 900, Coherent) for excitation at 400 nm and a polychromator with a CCD matrix for detection (LOT-Oriel, Instaspec IV). As reference for measurements of fluorescence quantum yield free base tetraphenylporphyrin (H<sub>2</sub>TPP) in toluene ( $\phi_{\rm fl} = 0.11$ ) [27] was used.

#### 2.3. Time resolved fluorescence

Time resolved fluorescence measurements of the samples dissolved in toluene were carried out by time-correlated-single-photon-counting-technique (Becker & Hickl GmbH, SPC 300) using frequency-doubled pulses of a Ti:Sapphire-Laser (Coherent Mira 900, 350–460 nm, FWHM 120 fs) for excitation. The response function of the system, measured with a fiber as scatter medium, had a FWHM of about 60 ps. The setup was previously described in [28].

# 2.4. Singlet oxygen generation

Photosensitized generated singlet oxygen luminescence (SOLM) of the molecules dissolved in toluene was measured time resolved at 1270 nm. A nanosecond Nd–YAG laser (BMI) together with an OPO (BMI) was used to excite the samples at 510 nm. The luminescence signal was recorded using a germanium pin diode (Northcoast). For calculation of the singlet oxygen quantum yield of both samples H<sub>2</sub>TPP in toluene was used as reference ( $\phi_{\Delta} = 0.68$ ) [18]. The setup and details are described elsewhere [29].

#### 3. Results and discussion

The structures of Ga-(OH)-TTP 1 and Ga-(OH)-chlorin 2 are shown in Fig. 1. The OH-group of 2 can be positioned either syn or anti to the 17-ester chain. Both isomers were not separated, since significant differences in the photophysical properties of both isomers are not expected. The absorption spectra of 1 and 2 are displayed in Fig. 2. Concerning the latter compound, it is well known that dihydrogenation of one of the peripheral cryptoolefinic double bonds of the porphyrin system causes pronounced changes in the electronic absorption spectrum. For both the chlorin and the metal porphyrin, shape and position of the absorption bands are the typical ones for compounds of the related class [1-5,8-10].

The reduced ring of a chlorin compared to the porphyrin ring system causes the characteristic, intense far-red shift of the  $Q_y$  (0, 0)-band (660 nm) in the absorption spectrum of Ga-(OH)-chlorin **2**, which is typical for chlorophyll related compounds [6]. Ga-(OH)-TTP **1** shows an intense and narrow Soret-band with an extinction of  $4 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$  at



Fig. 1. Structural formulas of the investigated compounds: hydroxy gallium (III)-porphyrin 1 and hydroxy gallium (III)-chlorin 2.



Fig. 2. Absorption spectra of Ga-(OH)-TTP 1 and Ga-(OH)-chlorin 2 in toluene.

424 nm compared to the Ga-(OH)-chlorin **2**, which has a broad Soret-band with an extinction of  $1 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ at 425 nm. Such absorption spectrum is typical for metal porphyrins [10]. The absorption spectrum of Ga-Cl-TPP which was investigated by Ohno et al. [3] had a similar shape. Due to the D<sub>4,h</sub>-symmetry the absorption spectrum of Ga-(OH)-TTP **1** has two Q-bands, as compared to the chlorin spectrum with four Q-bands (Fig. 1). The ratio between the intensity of the Soret-band and the Q<sub>y</sub> (0, 0)band of Ga-(OH)-chlorin **2** is two to one.

The S<sub>1</sub>-fluorescence spectra of both compounds consisted of two main bands (Fig. 3). For chlorin **2** the fluorescence peak at 666 nm is 10 times more intense than the peak at 725 nm. The opposite was found for the metal porphyrin, as the fluorescence intensity of the peak at 600 nm is about three times less intense than the second peak at 653 nm. In addition, the fluorescence spectrum of Ga-(OH)-TTP **1** shows a third fluorescence band at 426 nm, caused by emission from the S<sub>2</sub>-state (see inset in Fig. 3). The fluorescence spectrum of Ga-Cl-TPP published by Ohno et al. shows a similar shape as the Ga-(OH)-TTP **1** including the S<sub>2</sub>-fluorescence [3].

The photophysical parameters determined for both compounds are summarized in Table 1. Both compounds have a single exponential fluorescence decay with comparable lifetimes (Table 1). Otherwise, fluorescence quantum



Fig. 3. Fluorescence spectra of Ga-(OH)-TTP 1 and Ga-(OH)-chlorin 2 in toluene.

Table I				
Photophysical	parameters of (	Ga-(OH)-TTP 1	and Ga-(	OH)-chlorin 2

T-1-1- 1

	Ga-(OH)-TTP 1	Ga-(OH)-chlorin 2
B-band (nm)	424	425
Q-bands (nm)	553, 593	518, 560, 610, 660
$\lambda_{\rm em}$ (nm)	600, 653	666, 725
$S_2 - \lambda_{em}$ (nm)	426	_
$k_{\rm fl}  ({\rm s}^{-1})$	$2.4 \times 10^{7}$	$7.7 \times 10^{7}$
$k_{\rm ISC}  (\rm s^{-1})$	$2.47 \times 10^{8}$	$1.99 \times 10^{8}$
$\tau_{\rm fl}$ (ns)	2.43	2.87
$\phi_{\mathrm{fl}}$	0.06	0.22
$\phi_{\rm ISC}$	0.60	0.57
$\phi_{\Delta}$	0.60	0.57

yields ( $\phi_{\rm fl}$ ) differ significantly with 0.22 for Ga-(OH)-chlorin **2** and 0.06 for Ga-(OH)-TTP. Furthermore, the intersystem crossing quantum yield ( $\phi_{\rm ISC}$ ) is similar and high with 0.57 for the chlorin and 0.60 for the porphyrin, respectively. Because of similar fluorescence lifetimes and triplet quantum yields, an additional depopulation process of the S<sub>1</sub>-state occurring in the metal porphyrin can only explain these results. For the investigated Ga-(OH)-TTP **1**, a second deactivation process is the S<sub>2</sub>-fluorescence. The ratio between the intensity of the S<sub>2</sub>- and the S<sub>1,0</sub>-fluorescence (at 600 nm) is 1:89.

Both molecules have equal singlet oxygen and intersystem-crossing quantum yields. This behavior is typical for tetrapyrroles [30]. The fact that both compounds have a high  $\phi_{\rm ISC}$  and that nearly all molecules in the triplet state are deactivated by energy transfer to molecular oxygen, generating molecular singlet oxygen, makes these compounds interesting for PDT.

# 4. Conclusions

The photophysical properties of both compounds make them interesting for application as photosensitizers in PDT and as electron donors in artificial photosystems. Moreover the S<sub>2</sub>-fluorescence of the Ga-(OH)-TTP could be used as strong measure of the efficiency of light induced electron transfer in artificial photosystems. The Ga-(OH)chlorin has suitable absorption bands and extinction coefficients as well as a high singlet oxygen quantum yield to be effectively utilized as photosensitizer in PDT. It might possibly be employed favorably in combination with ultrasonic sound therapy, which is based on the unique sensitizing properties of certain gallium containing compounds. Compared to the porphyrin, the considerable bathochromic shift of the Q absorption bands together with the stronger absorption of the chlorin enables the use of light with longer wavelengths, increasing the depth of tissue penetration, and leading to more efficient tumor damage. Finally, the fifth axial coordination site of gallium in these porphyrins and chlorins make them potentially useful for functionalization of the macrocycle with suitable moieties in order to obtain more complex chromophor arrays, e.g., gallium containing dyads for photoinduced electron transfer studies. Importantly, the axial approach bears the advantage that the macrocycle preserves its electronic characteristics.

#### Acknowledgments

The authors thanks Mr. Wöhlecke for the technical support.

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