

## Formation of Associates of Porphyrins and Water in $C_6D_6$ Medium

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Using the DOSY method, the equilibrium constants ( $K_c$ ) for the formation–decomposition of molecular associates for a number of porphyrins and chlorins with a water molecule in the  $C_6D_6$  medium were determined.  $K_c$  and the proportion of porphyrin-bound water were determined experimentally from the dependences of the self-diffusion coefficients  $D$  on the diffusion time  $t_m$ ,  $D(t_m)$ . The formation of water-porphyrin associates is a necessary condition for proton exchange between porphyrin and water in the  $C_6D_6$  medium. It was established that the ability of the studied macroheterocyclic compounds to form associates with water molecules due to the formation of intermolecular hydrogen bonds, as a rule, increases with an increase in the deviation from the planarity of the macrocycle and, consequently, an increase in the steric accessibility of intracyclic NH groups and nitrogen atoms. The obtained data indicate that the  $K_c$  value in the absence of substituents capable of forming hydrogen bonds in the studied macroheterocyclic compounds can be used as an independent criterion for assessing the nonplanarity of the macrocycle in addition to the currently existing structural, spectral and computational methods, as well as to the data obtained in the study of other model reactions.

**Keywords:** Porphyrins, DOSY spectroscopy, proton exchange, associates, deuteroporphyrin IX, tetraphenylporphine, methylpyropheophorbide *a*, chlorin *e*<sub>6</sub>.

## Образование ассоциатов порфиринов и воды в среде $C_6D_6$

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Методом DOSY определены константы ( $K_c$ ) равновесия образования-распада ассоциатов молекул ряда порфиринов и хлоринов с молекулой воды в среде  $C_6D_6$ .  $K_c$  и доля связанной с порфирином воды определены экспериментально из зависимостей коэффициентов самодиффузии  $D$  от времени диффузии  $t_m$ ,  $D(t_m)$ . Показано, что образование ассоциатов «вода-порфирин» является необходимым условием протонного обмена между порфирином и водой в среде  $C_6D_6$ . Установлено, что способность исследованных макрогетероциклических соединений к формированию ассоциатов с молекулами воды за счет образования межмолекулярных водородных связей, как правило, повышается с ростом отклонения от планарности макроцикла и, следовательно, стерической доступности внутрициклических групп NH и атомов азота. Полученные данные свидетельствуют о том, что величина  $K_c$  при отсутствии у исследуемых макрогетероциклических соединений заместителей, способных образовывать водородные связи, может быть использована в качестве независимого критерия оценки непланарности макроцикла в дополнение к существующим структурным, спектральным и расчетным методам, а также данным изучения других модельных реакций.

**Ключевые слова:** Порфирины, спектроскопия DOSY, протонный обмен, ассоциаты, дейтеропорфирин IX, тетрафенилпорфин, метилпирофеофорбид *a*, хлорин *e*<sub>6</sub>.

## Introduction

The deviation of aromatic macrocycles of porphyrins and their analogs from a planar structure significantly affects the chemical, spectral and other properties of these compounds.<sup>[1–6]</sup> Therefore, the assessment of the nonplanarity of porphyrin macrocycles is of considerable interest.<sup>[1]</sup> To assess the nonplanarity of a porphyrin macrocycle, several mutually complementing groups of methods are used: direct structural methods (X-ray diffraction analysis (XRD) and electron diffraction), spectral methods (fluorescence spectroscopy, resonance Raman spectroscopy, <sup>1</sup>H NMR spectroscopy, *etc.*), as well as various computational methods.<sup>[1]</sup> Moreover, to assess the nonplanarity of a macrocycle, data on the reactivity of the coordination center of the macrocycle are widely used (for example, in reactions of complex formation, acid dissociation of intracyclic NH groups, *etc.*).<sup>[1]</sup> The formation of an associate of porphyrin with water that involves the formation of intermolecular hydrogen bonds of intracyclic NH groups and nitrogen atoms of the macrocycle can be used as a model reaction to assess the nonplanarity of the porphyrin macrocycle. The strength of the porphyrin-water hydrogen bond should depend on the steric accessibility of the intracyclic NH groups and nitrogen atoms, which, in turn, depends on the degree of distortion of the porphyrin macrocycle (the stronger the deviation from the planar structure, the more sterically accessible the NH groups and nitrogen atoms). Therefore, the values of the equilibrium constants for the formation – decomposition of the porphyrin-water associate can be used for comparison of the nonplanarity of various porphyrins. We have previously shown that proton exchange between water molecules and various natural and synthetic porphyrins in the CDCl<sub>3</sub> and C<sub>6</sub>D<sub>6</sub> medium is manifested in DOSY by an increase in the self-diffusion coefficients *D* of protons of the intracyclic NH group (*D*<sub>NH</sub>) in comparison with the C-H protons of the porphyrin molecule (*D*<sub>CH</sub>).<sup>[7–9]</sup> As we found out, the constants of the rate of transfer of the intracyclic proton of the NH group of porphyrin to a water molecule (*k*<sub>NH</sub>) and the reverse process (*k*<sub>w</sub>) for dimethyl ester of deuterioporphyrin IX in CDCl<sub>3</sub><sup>[8]</sup> and for a number of natural and synthetic porphyrins in C<sub>6</sub>D<sub>6</sub><sup>[9]</sup> differ from each other, with *k*<sub>w</sub> always exceeding *k*<sub>NH</sub>. The disparity between the *k*<sub>NH</sub> and *k*<sub>w</sub> values is explained by the formation of water-porphyrin associates due to intermolecular hydrogen bonds with the participation of intracyclic nitrogen atoms of porphyrin, resulting in a decrease in the self-diffusion coefficients of water molecules *D*<sub>w</sub>,

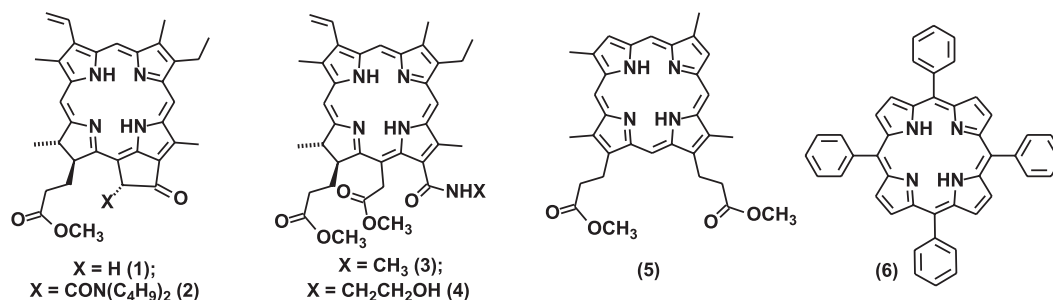
what takes place not only due to proton exchange, but also due to the fact that for a fraction of the time water slowly diffuses as part of the associate.<sup>[7,8]</sup> According to the conceptions of the mechanism of proton exchange generalized in the literature,<sup>[10]</sup> without the formation of a porphyrin-water associate, the implementation of proton exchange is impossible; therefore, the study of the formation of such associates makes it possible to obtain information that is of interest not only from the point of view of assessing the nonplanarity of the macrocycle, but also from the point of view of describing proton exchange as a whole. In this work, using the DOSY method, we have registered the formation of porphyrin-water associates for a number of natural and synthetic porphyrins **1–6** (Scheme 1) and determined the equilibrium constants for these processes.

## Materials and Methods

Methylpyropheophorbide *a* (**1**), deuterioporphyrin dimethyl ester (**5**) and *meso*-tetraphenylporphyrine (**6**) were obtained according to <sup>[11]</sup>. Dibutylamide (**2**),<sup>[12]</sup> chlorin *e*<sub>6</sub> methylamide (**3**),<sup>[13]</sup> ethanolamide (**4**)<sup>[14]</sup> were obtained according the known procedures. The spectral characteristics of the studied compounds correspond to the literature data.<sup>[8]</sup> The solutions under study were sealed in standard Bruker NMR ampoules with a diameter of 5 mm. All experiments performed with these samples showed that the samples are stable for at least 2 years. The measurements were performed on a Bruker Avance II spectrometer (operating frequency 300 MHz). NMR and DOSY spectra were processed using the MestReNova 14.2.1–27684 program. The concentration of the compounds under study in C<sub>6</sub>D<sub>6</sub> was 0.005–0.010 M. The concentration of water in C<sub>6</sub>D<sub>6</sub> was 0.01 M. Parameters of the experiment were as follows: the pulse sequence “double stimulated echo with bipolar gradient pulses” was implemented on a Bruker Avance II spectrometer using the standard *dstebpgp3s* program with convection compensation; the duration of diffusion *D*20 varied from 0.025 s to 1 s; the duration of gradient pulses *P*30 varied from 150 to 1,500 μs; the accumulation number *NS* = 64; the number of gradient spectra *td* (*F1*) = 16; each spectrum has a sampling frequency of 16k. Time *D*1 = 5 s (relaxation times for all measured lines of the NMR spectrum did not exceed 1.8 s). The experiments were carried out with temperature stabilization (25 °C) using an air cushion to reduce the influence of external vibrations. The air flow rate in the thermostat was 670 L/h.

## Results and Discussion

As noted above, one of the pathways of interaction of porphyrin and water in C<sub>6</sub>D<sub>6</sub> medium is the reversible



Scheme 1.

formation of a porphyrin-water associate according to schematic reaction (1).



In a solution for the reversible association-dissociation reaction described by (1), a dynamic equilibrium is established, so that the amount of the formed water-porphyrin (WP) associates and, accordingly, the proportion of porphyrin-bound water will be constant over time. The value of the equilibrium concentration of water-porphyrin associates  $[\text{WP}]_\infty$  can be determined from the condition of dynamic chemical equilibrium:

$$\frac{\partial[(\text{WP})]}{\partial t} = k_1 \cdot ([\text{W}_0] - [(\text{WP})]) \cdot ([\text{P}_0] - [\text{WP}]) - k_2 \cdot [(\text{WP})] = 0, \quad (2)$$

where  $k_1$  and  $k_2$  are the rate constants of the forward and reverse reaction (1),  $[\text{W}_0]$  and  $[\text{P}_0]$  are the equilibrium concentrations of water and porphyrin in the solution,  $[\text{WP}]$  is the concentration of the water-porphyrin associate in the solution.

Then the expression for the equilibrium constant can be written as follows:

$$K_c = \frac{k_1}{k_2} = \frac{[\text{WP}]_\infty}{([\text{W}_0] - [\text{WP}]_\infty) \cdot ([\text{P}_0] - [\text{WP}]_\infty)} \quad (3)$$

The formation of the porphyrin-water associate should affect the average over the observation time self-diffusion coefficient  $D$  of water protons ( $D_w$ ). Since for a fraction of the time the water molecule moves as part of the associate,  $D_w$  should decrease in comparison with  $D$  of water molecules in the absence of porphyrin in the solution, and the more time the water molecule spends as part of the associate (that is, the more stable the associate), the more  $D_w$  decreases. The change in  $D$  can be measured by the DOSY method. However, the detection of the porphyrin-water associate by the DOSY method is complicated by the fact that  $D_w$  changes not only during the formation of the associate itself, but also due to the participation of water in proton exchange. It is known<sup>[7,8,15–17]</sup> that the  $D$  value of exchanging protons depends on the time of diffusion observation  $t_m$  ( $D_{20}$ ). If the change in  $D_w$  was associated only with the exchange, then at  $t_m = 0$   $D_w$  should coincide with the value of the diffusion coefficient of free water  $D_{wF}$  ( $D_{wF}$  is  $D$  for water in  $\text{C}_6\text{D}_6$  without porphyrin), since the exchange did not yet occur. Nevertheless, a noticeable difference between the values of  $D_w(0)$  and  $D_{wF}$  was observed at  $t_m$  close to zero.<sup>[7,8]</sup> This difference may be a result of the formation of porphyrin-

water associates. In practice, DOSY measurements at  $t_m = 0$  are impossible for technical reasons associated with imperfect parameters of radio frequency and gradient pulses. The minimum possible value for the available equipment was  $t_m \approx 0.02$  s. However, the extrapolation of the  $D_w(t_m)$  dependences allows to obtain sufficiently accurate  $D_w(0)$  values for all measured samples.

Since porphyrin-associated water molecules have a self-diffusion coefficient that coincides with the self-diffusion coefficient  $D_p$  for porphyrin, the average observed self-diffusion coefficient of water  $D_w(0)$  can be expressed by the relation:

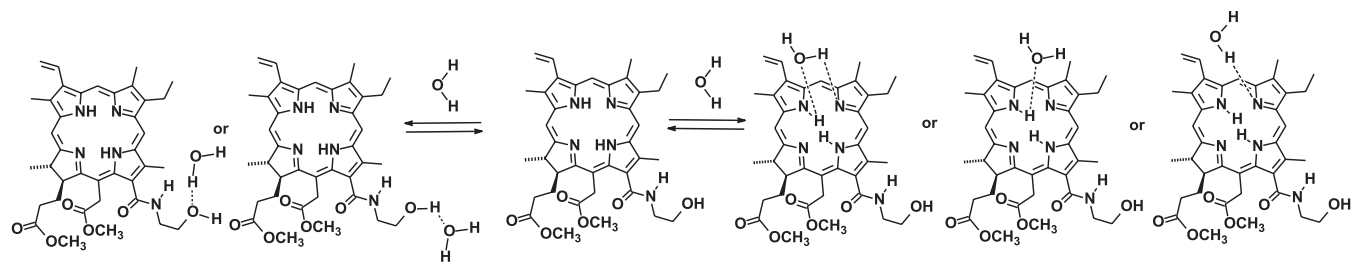
$$D_w(0) = D_{wF} \cdot (1 - b_w) + D_p \cdot b_w, \quad (4)$$

where  $D_{wF}$  is the self-diffusion coefficient of water in benzene in the absence of porphyrin,  $b_w$  is the fraction of the associated water. Thus, if we know  $b_w$ , we can find, and then from expression (3) we can determine the equilibrium constant  $K_c$ . The values of  $K_c$  obtained in this way are presented in Table 1. The  $^1\text{H}$  NMR and DOSY spectra are provided in the Supporting materials.

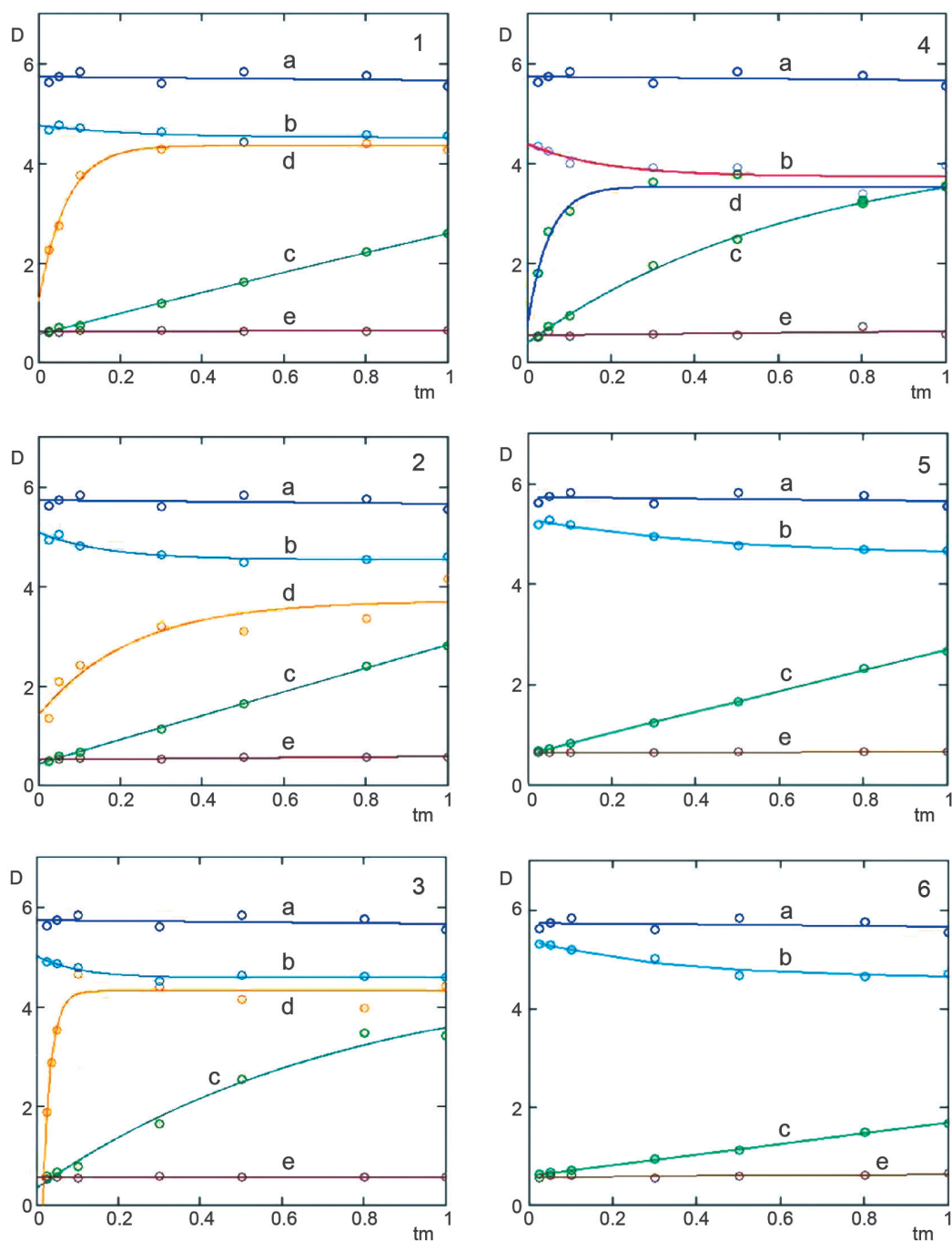
The porphyrins and chlorins **1–6** studied in this work differ from each other in the degree of nonplanarity of the macrocycle both due to the hydrogenation of one of the pyrrole rings (chlorins **1–4** and porphyrins **5, 6**) and, for compounds with a chlorin macrocycle, due to the presence (compounds **1** and **2**) or the absence (compounds **3** and **4**) of an exocycle. Compound **4**, in addition, has a hydroxyl group at the periphery of the macrocycle, which should also affect its ability to bind water molecules. In accordance with the  $K_c$  values determined in this work (Table 1), the studied compounds can be divided into two groups. The lowest  $K_c$  values are observed for porphyrins **5** and **6**, what corresponds to a greater planarity of the macrocycle and, accordingly, a lower steric accessibility of intracyclic NH groups and nitrogen atoms of the macrocycle in comparison with chlorins **1–4**. Despite the different nature of substitution in macrocycles **5** and **6**, the  $K_c$  values within the error limits do not differ. In most cases, hydrogenation of one of the pyrrole rings in chlorins **1–4** leads to an increase in their ability to bind water, which is expressed in a significant increase in the  $K_c$  value. The exception is dibutylamide **4**, the  $K_c$  value of which (although it is somewhat higher than that of porphyrins **5** and **6**), contrary to expectations, is closer to porphyrins than to chlorins **1–3**. This may be due to the influence of peripheral substituents incapable of forming hydrogen bonds on the interaction with water. The reason for this influence is not clear. Apparently, intermolecular interactions in a solution of this compound have a more complex nature. The same reason may be respon-

**Table 1.** Equilibrium constants ( $K_c$ ) of porphyrin-water associates formation for compounds **1–6** in  $\text{C}_6\text{D}_6$  medium at 25 °C.

Compound	$K_c$ ( $\text{L} \cdot \text{mol}^{-1}$ )	Compound	$K_c$ ( $\text{L} \cdot \text{mol}^{-1}$ )
<b>5</b>	0.32±0.03	<b>1</b>	2.1±0.2
<b>6</b>	0.33±0.03	<b>3</b>	2.2±0.2
<b>2</b>	0.41±0.04	<b>4</b>	4.4±0.4



Scheme 2



**Figure 1.** Experimental and calculated dependences of the self-diffusion coefficients  $D$  ( $1 \cdot 10^{-9} \text{ m}^2/\text{s}$ ) on the diffusion time  $t_m$  (s) for compounds **1–6**. a –  $D_{WF}$  is the self-diffusion coefficient for free water; b –  $D_w$  is the self-diffusion coefficient for the NMR line of water; c –  $D_{NH}$  is for NMR lines of intracyclic NH groups (both equivalent groups for compounds **5** and **6** and I-NH for compounds **1–4**); d –  $D_{NH}$  is for NMR lines of intracyclic groups III-NH (for compounds **1–4**); e –  $D_p$  is the self-diffusion coefficient of protons of carbon atoms of deuterioporphyrin.



sible for the fact that very similar  $K_c$  values are observed for chlorin **1** that has an exocycle and chlorin **3** that does not have an exocycle. In the case of chlorin **4** that has an OH group capable of forming hydrogen bonds at the periphery of the macrocycle,  $K_c$  was significantly higher, which indicates the possibility of binding a water molecule by such peripheral substituents (Scheme 2). A significant increase in  $K_c$  in the case of chlorin **4** in comparison with chlorin **3** that is similar in the structure of the macrocycle and peripheral substituents are also consistent with the significantly higher steric accessibility of the hydroxyl group compared to the intracyclic NH groups and nitrogen atoms.

## Conclusion

In this work, we used the DOSY method to determine the equilibrium constants ( $K_c$ ) of the formation-decomposition of molecular associates of a number of porphyrins and chlorins with a water molecule in the  $C_6D_6$  medium.  $K_c$  and the proportion of porphyrin-associated water were experimentally determined from the dependences of the self-diffusion coefficients  $D$  on the diffusion time  $t_m$ ,  $D(t_m)$ . We showed that the formation of water-porphyrin associates is a necessary condition for proton exchange between porphyrin and water in the  $C_6D_6$  medium. We established that the ability of the studied macroheterocyclic compounds to form associates with water molecules due to the formation of intermolecular hydrogen bonds, as a rule, increases with an increase in the deviation from the planarity of the macrocycle and, consequently, in the steric accessibility of intracyclic NH groups and nitrogen atoms.  $K_c$  for derivatives with a porphyrin macrocycle is always lower than for derivatives with a chlorin macrocycle. The introduction to the periphery of the macrocycle of a hydroxyl group which is not only capable of forming hydrogen bonds but is also sterically more accessible in comparison with intracyclic NH groups and nitrogen atoms results in a sharp increase in  $K_c$ . The data obtained indicate that the  $K_c$  value in the absence of substituents capable of forming hydrogen bonds in the studied macroheterocyclic compounds can be used as an independent criterion for assessing the nonplanarity of the macrocycle in addition to the currently existing structural, spectral and computational methods, as well as to the data obtained in the study of other model reactions.

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