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Molecular Complex Formation Between Gadolinium(III) Tetraphenylporphyrin and Vitamin E

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Summary. The complex formation between Gd(III) tetraphenylporphyrin or free-base tetraphenylporphyrin and vitamin E have been studied by spectrophotometric titration in chloroform and cyclohexane solutions. It has been shown that tetraphenylporphyrin and its gadolinium complex form 1:1 molecular complexes with vitamin E. The absorption spectra of titrated porphyrins contain well defined isosbestic points. The equilibrium constants were found using curve fitting procedure. The observed interactions are stronger for metallated than for non-metallated porphyrin and in less polar than in polar solvents.

Keywords. Gadolinium; Interaction; Porphyrin; Vitamin E.

Ausbildung von Molekülverbindungen zwischen Gadolinium(III)-tetraphenylporphyrin und Vitamin E

Zusammenfassung. Die Ausbildung von Molekülverbindungen zwischen Gd(III)-Tetraphenylporphyrin oder der freien Base und Vitamin E wurden mittels spektrophotometrischer Titration in Cyclohexan und Chloroform untersucht. Es wird gezeigt, daß Tetraphenylporphyrin und sein Gadoliniumkomplex mit Vitamin E Molekülverbindungen äquimolarer Zusammensetzung bilden. Die Absorptionsspektren der titrierten Porphyrine zeigen gut definierte isosbestische Punkte. Die Gleichgewichtskonstanten wurden unter Zuhilfenahme eines *curve-fitting*-Algorithmus ermittelt. Die beobachteten Wechselwirkungen sind stärker für metalliertes als für nichtmetalliertes Porphyrin und in unpolaren als in polaren Lösungsmitteln.

Introduction

Interest in the chemistry of lanthanide porphyrin complexes has been growing since their successful synthesis in 1976 [1]. These compounds have been applied as non destructive probes inserted into molecules of biological interest [2, 3]. Gadolinium(III)porphyrins have attracted particular attention as in these complexes the tumor concentration ability of porphyrins [4, 5] combines with the special properties of the f^7 electronic configuration of gadolinium (III). Paramagnetic Gd^{3+} ions incorporated into the porphyrin moiety may be used as a contrast agent in

NMR or EPR imaging processes [6]. The possibility of employing Gd(III) hematoporphyrin as a tumor diagnostic localizer and at the same time as a sensitizer for the photoradiation therapy has been recently described [7].

Contrary to the complexes of other metalloporphyrins, the coordination chemistry of lanthanide porphyrin in solutions is a rather unexplored area [8, 9]. Especially many aspects of the transition metal-porphyrin coordination chemistry have been investigated [10].

It is well established that porphyrins and metalloporphyrins form 1:1 molecular complexes with electron acceptors and donors [11–13]. Theoretical analysis [14] of the electronic structure of the porphyrins demonstrates that porphyrins can exhibit ampholytic character. The carbon atoms of the pyrrole ring should be good electron donors as they have an excess of π-electrons. On the other hand, the carbon atoms in the methene bridges are electron deficient and could be good electron acceptors. Formation of these donor-acceptor (*charge transfer*) molecular complexes involving porphyrins play pivotal roles in many processes of biological importance [13, 15–17]. According to the literature, the interaction of vitamin E has not yet been investigated, neither with Gd(III) tetraphenylporphyrin (Gd*TPP*) nor with free-base tetraphenylporphyrin (H_2*TPP*).

This work was undertaken to control the formation of the molecular complex between Gd*TPP* and vitamin E and to estimate the force of this usually weak interaction by calculation of the apparent stability constants in two solvents with different polarity: chloroform and cyclohexane. To understand how the complex formation is affected by the gadolinium(III) inserted into the porphyrin moiety, the Gd*TPP*(*acac*)-vitamin E interaction is compared with the H_2*TPP*-vitamin E interaction.

Results and Discussion

The solutions of tetraphenylporphyrin and its gadolinium complex in chloroform or cyclohexane in the concentration range of 10^{-6}–10^{-5} mol·dm^{-3} were spectrophotometrically titrated with 0.2 *M* vitamin E solutions in chloroform or cyclohexane, respectively. The porphyrins obey *Beer-Lambert's* law in this concentration range [25]. Figs. 1 and 2 are examples of the spectral changes upon titration. The spectra shown were recorded in one spectrophotometrical cell. For illustration, some part of the spectra were enlarged or expanded. Vitamin E does not show any band neither in the *Soret* nor in the Q-band region of the porphyrin spectra. Fig. 1 shows the UV-Vis spectral changes that occur upon titration of a chloroform solution of H_2*TPP* with a vitamin E solution in chloroform. The B-band of the starting solution at 417 nm was not significally shifted, only diminished in intensity. The same was observed with all four components of the Q-band. However, the evolution of the spectra is different from that which usually occurs during diluting the porphyrin solutions, as ten sharp isosbestic points (two for each peak) were present at 412, 431, 512, 530, 548, 566, 596, 612, 644, and 676 nm.

Evolution of the absorption spectra for the Gadolinium(III) tetraphenylporphyrin titration with vitamin E is displayed in Fig. 2. The B-band of starting the solution at 426 nm was red-shifted into 428 nm, and its intensity increased. Three sharp isosbestic points were observed at 414.2, 426.5, and 437.8 nm.

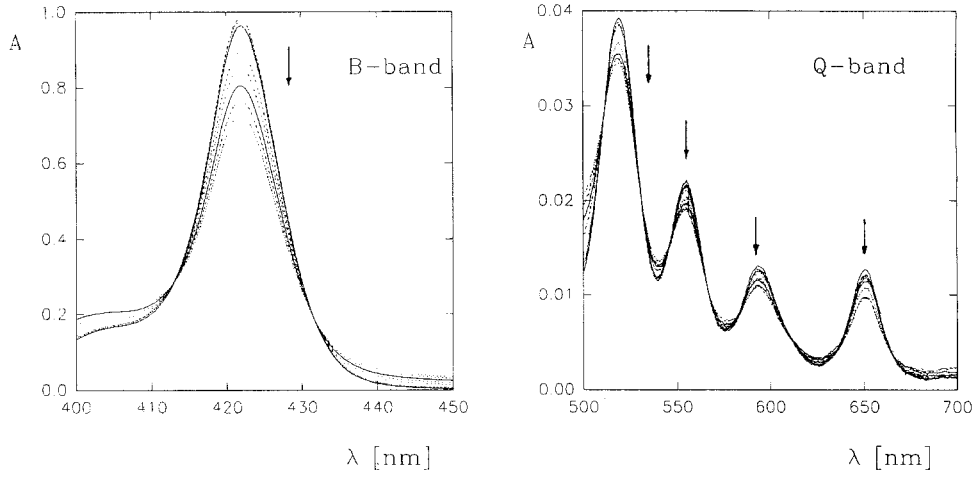


Fig. 1. Evolution of the absorption spectra upon titration of a $2.3 \cdot 10^{-6}$ *M* chloroform solution of H_2TPP with 0.24 *M* vitamin E in chloroform

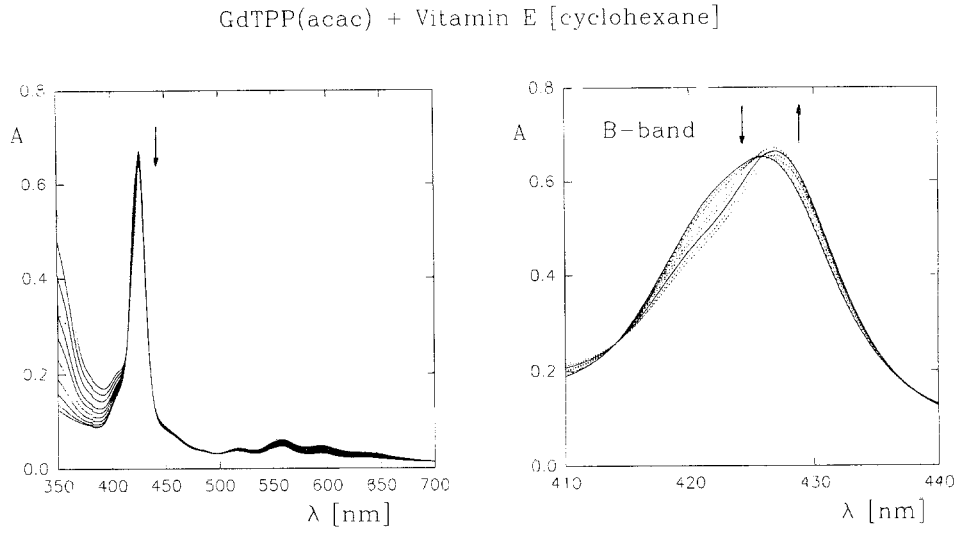


Fig. 2. Evolution of the absorption spectra upon titration of a $3.1 \cdot 10^{-6}$ *M* cyclohexane solution of Gd*TPP*(*acac*) with 0.19 *M* vitamin E in cyclohexane

The evolution of the porphyrin spectra upon titration with vitamin E indicated that a process of molecular complex formation has taken place; however, interactions were very weak. Formation of new bands was not observed. To find definitive a answer if interactions between porphyrins and vitamin E occur, the observed absorbance change at the *Soret* band during titration with vitamin E was plotted against the molar concentration of the porphyrins and compared with the absorbance calculated using *Beer-Lambert's* law. The calculated plot shows how absorbance would change with the assumption that interaction does not take place and only dilution effects are observed. These plots were compared with identical

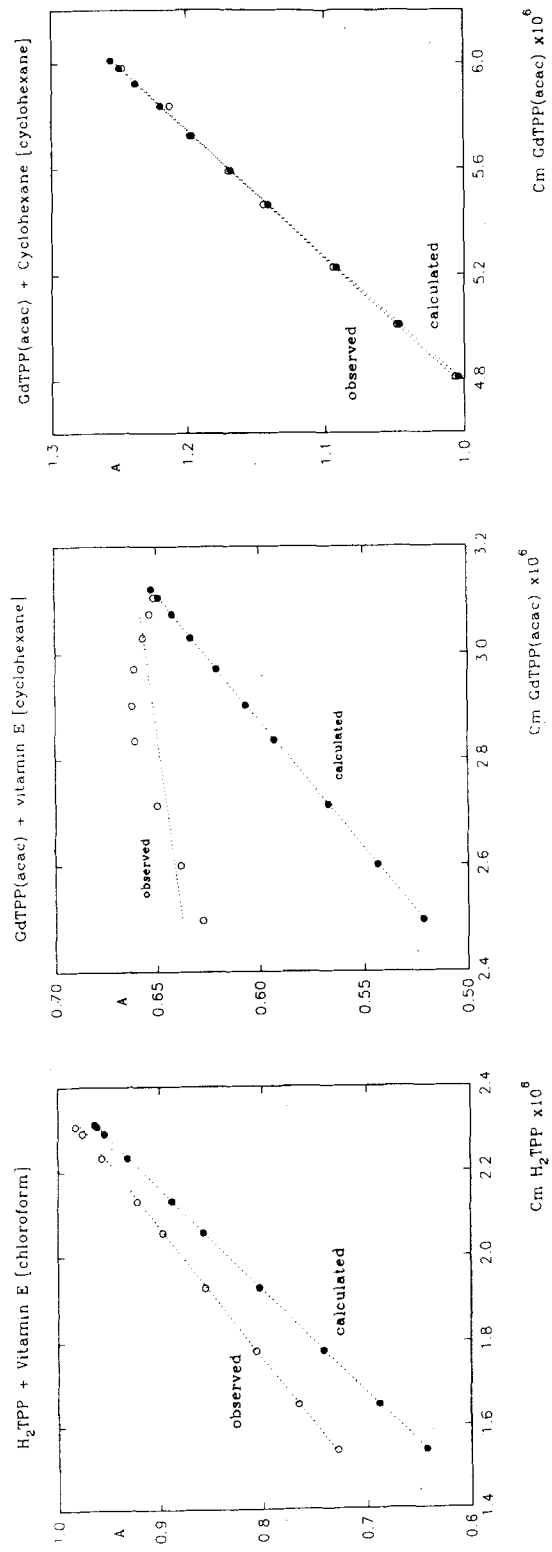


Fig. 3. Plot of the absorbance change at the *Soret* band upon titration with vitamin E (molecular complex formation) and with pure solvent titration (no interaction) *vs.* C_M of porphyrin (○ – observed points, ● – calculated from *Beer-Lambert's* law)

plots for titrations with pure solvents. Examples are shown in Fig. 3. The linearity of the plots was controlled using a linear regression routine. The corresponding data are presented in Table 2. In all cases where interactions are absent the slopes of the observed and calculated straight lines are almost identical; Linear regression coefficients (R^2) for interacting pairs of compounds are lower.

The experimental results presented here prove the formation of a complex between vitamin E and H_2TPP and Gd*TPP(acac)* in both chloroform and cyclohexane. The conclusion is supported by the presence of isosbestic points and the deviation from linearity in *Beer-Lambert's* law plots. However, the small shift of the *Soret* band and the absence new band formation suggest that interactions are weak. Vitamin E is commonly considered as antioxidant and in many processes plays the role of an electron donor [26].

For the interaction of porphyrin (P) with vitamin E (V) in equilibrium given by equation (1),

$$P \xrightarrow{V} PV \xrightarrow{V} PV_2 \cdots \xrightarrow{V} PV_n \tag{1}$$

the equilibrium constants (K_i) can be written as

$$K_i = \frac{[PV_i]}{[PV_{i-1}][V]} \tag{2}$$

Among several methods available in literature to calculate the values of association constants [27], the most popular is the *Benesi-Hildebrandt* method [15, 20, 27, 28]. Applying this method to our system is not possible as the absorption bands of the formed complex are located at the same wavelength as those of porphyrin.

To evaluate the stoichiometry and the magnitude of the vitamin E and porphyrin binding constants (K), the expression described by *Beck* [23] was used for the determination of stability constants in the case of successive complex formation:

$$A = \frac{\varepsilon_0 + \varepsilon_1 \cdot K_1 \cdot [V] + \varepsilon_2 \cdot K_2 \cdot [V]^2 + \cdots + \varepsilon_n \cdot K_n \cdot [V]^n}{1 + K_1 \cdot [V] + K_1 \cdot K_2 \cdot [V]^2 + \cdots + K_1 \cdot K_2 \cdot \cdots \cdot K_n \cdot [V]^n} [P_0] \tag{3}$$

where A is the absorbance, ε_0 the molecular absorbance coefficient for porphyrin, ε_1 and K_1, ε_2 and K_2,..., *etc.* are the molecular absorbance coefficients and stability constants for complexes with the stoichiometry 1:1, 1:2,..., *etc.*, and [V] and $[P_0]$ are the analytical concentrations of vitamin E and porphyrin with the assumption that $[V] \gg [P_0]$.

To calculate K, the experimental data were fitted to equation (3) using the non-linear fitting procedure based on the *Marquardt-Levenberg* algorithm (program SigmaPlot) [24]. The fitting was performed for the complexes with the stoichiometry 1:1 and 1:2, but only results for 1:1 complexes gave a good accordance between the calculated curve and the experimental points. An example of fitting is shown in Fig. 4 where experimental points cover very well the theoretical curve generated from equation (3) for 1:1 complexes. The values of the association constants K for the analyzed complexes are given in Table 2. The association constants are low comparable with literature data [15], suggesting a rather weak interaction.

The interactions between Gd*TPP(acac)* and vitamin E are stronger than those between H_2TPP and vitamin E in both solvents. This indicates that the metal is

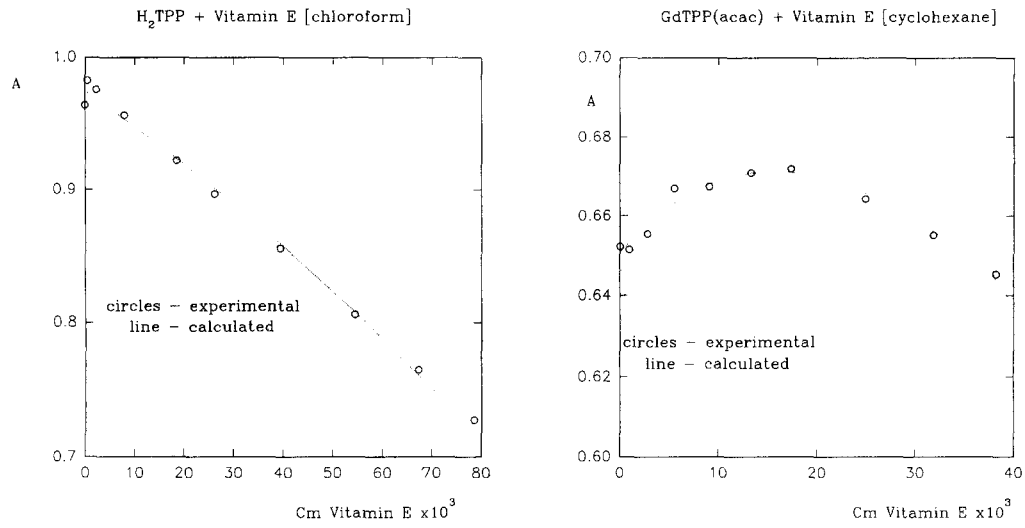


Fig. 4. Plot of the absorbance change at the *Soret* band upon titration with vitamin E *vs.* C_M of vitamin E. Circles are experimental points. The theoretical curve was generated from equation (3)

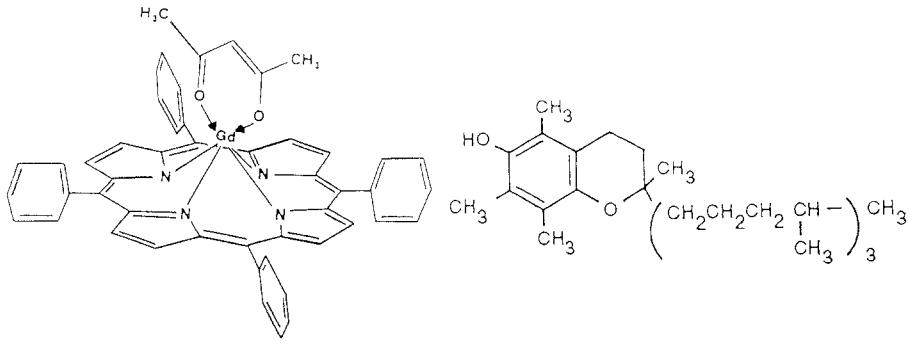
involved in complex formation. On the other hand, it is difficult to expect that vitamin E could enter the gadolinium coordination sphere, even with the "tail" side of the molecule. However, the additional coordination of the gadolinium(III) ion, which is coordinatively unsaturated in the porphyrin complex, can not be entirely excluded. The process of axial ligand exchange must be excluded as the expected values of K for such a processes are much higher. The presence of the metal in the porphyrin moiety probably alters the electron distribution and facilitates *charge transfer* processes.

The complexes formed in cyclohexane have higher association constants than complexes in chloroform. Chloroform is the more polar solvent; stronger solvent effects probably make *charge transfer* processes more difficult. The stronger interactions in cyclohexane also suggest stronger interactions in fatty tissues, where vitamin E is commonly soluble. This observation may be significant for the insertion of gadolinium as a magnetical probe into material of biological importance.

Experimental

Materials. 5, 10, 15, 20-Tetraphenyl-21*H*, 23*H* porphyrin and vitamin E (*DL*-α-tocopherol, see Scheme 1) were obtained from Aldrich, gadolinium(III) acetylacetonate hydrate from Strem Chemicals, and 1, 2, 4,-trichlorobenzene from Merck. All substances were used without further purification. Neutral Al_2O_3 (activity 90, 70–230 mesh, Merck) was used for column chromatography. All solvents used were of analytical grade (SDS – Solvants, Documentation, Synthese) and were kept over molecular sieves (3 Å).

Synthesis. The tetraphenyl porphyrin complex of gadolinium Gd(III)*TPP*(*acac*), where H_2TPP = tetraphenylporphyrin and H*acac* = acetylacetone (see Scheme 1), was prepared by the method described originally by *Horrocks* and *Wong* [1, 18, 19] and later modified by other authors [20, 21]. A mixture of hydrated Gd(*acac*)$_3$ (150 mg) and porphyrin (50 mg) in 1,2,4-trichlorobenzene was heated under reflux in an argon atmosphere for 4 hours. The course of the reaction was followed by UV-Vis



Scheme 1

Table 1. Band centers in the absorption spectra of the tetraphenylporphyrin and Gd(III) tetraphenylporphyrin complexes

Compound	Solvent	λ [nm] ($\log \varepsilon$)
Gd*TPP*(*acac*)	toluene	421 (5.39) 515 (3.50) 555 (4.07) 593 (3.57)
	chloroform	425 (5.43) 517 (3.48) 553 (4.12) 591 (3.64)
	cyclohexane	426 (5.32) 517 (3.46) 556 (4.04) 593 (3.52)
H_2TPP	chloroform	417 (5.62) 515 (4.22) 552 (3.95) 594 (3.70) 650 (3.60)
	cyclohexane	420 (5.62) 517 (4.17) 550 (3.85) 595 (3.66) 655 (3.54)

Table 2. Comparison of the deviation from linearity of the observed and calculated absorbance plot *vs.* C_M of porphyrins and values of the association constants K

Compound	Slope observed	R^2 observed	Slope calculated	R^2 calculated	K [$dm^3 \cdot mol^{-1}$]
(in cyclohexane)					
H_2TPP + cyclohexane	0.39576	0.99989	0.40951	0.99975	no interaction
H_2TPP + vitamin E	0.36127	0.99945	0.41679	1.00000	8.1 ± 1
Gd*TPP*(*acac*) + cyclohexane	0.20496	0.99957	0.20895	1.00000	no interaction
Gd*TPP*(*acac*) + vitamin E	0.34960	0.70761	0.20843	1.00000	15.6 ± 2
(in chloroform)					
H_2TPP + chloroform	0.41759	0.99894	0.41532	0.99993	no interaction
H_2TPP + vitamin E	0.32375	0.99881	0.41689	1.00000	2.4 ± 2
Gd*TPP*(*acac*) + chloroform	0.27333	0.99979	0.26752	0.99994	no interaction
Gd*TPP*(*acac*) + vitamin E	0.13699	0.99552	0.26930	1.00000	3.7 ± 1

absorption spectroscopy. After completion of the reaction, the 1.2,4-trichlorobenzene was removed by evaporation under reduced pressure. The solid residue was vacuum dried overnight, dissolved in CH_2Cl_2 and applied to the top of a neutral Al_2O_3 column. The unreacted H_2TPP was eluted first with toluene and then with a mixture of toluene and MeOH (98:2 v/v). The pure Gd*TPP*(*acac*) (purity was checked by UV-Vis absorption spectroscopy and TLC) was eluted with dimethylsulfoxide (*DMSO*) followed by extraction of gadolinium porphyrin from the eluate with chloroform. The solvent was removed by vacuum evaporation. The final product was obtained as an amorphous powder. Anal.:

calc. for Gd*TPP*(*acac*)·6H_2O ($GdC_{49}H_{47}N_4O_8$): C, 60.23; H, 3.61; N, 5.73%; found: C_1 59.88; H, 3.81; N, 5.29%. The UV-Vis absorption spectra are documented in Table 1.

Measurements. Absorption spectra were recorded wth a Perkin Elmer Lambda 7 spectrophotometer, using 1 cm quartz cells, in the 300–700 nm region at a temperature of 21 ± 1°C. Spectra were stored on disk under control of the program PECSS (Perkin Elmer) and converted to ASCII files using the JCAMP option. The database program SigmaPlot was used for manipulating and plotting the data [22].

Method. Equilibrium constants for vitamin E binding to H_2*TPP* and Gd*TPP*(*acac*) were determined by the spectrophotometric titration procedure in a covered 10 mm spectrophotometric cell equipped with a magnetic stirrer. The vitamin E solutions in chloroform or cyclohexane were added in increments to 2 ml chloroform or cyclohexane solutions of porphyrin with a *Hamilton* syringe until no further change in the spectrum was observed. In parallel experiments, pure solvents were added in the same increments to solutions of porphyrins of the same concentrations. Apparent stability constants were calculated using the *Beck* equation [23]. The equation was fitted with a non-linear regression procedure based on the *Marquardt-Levenberg* algorithm using the SigmaPlot program [24].

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References

[1] Horrocks D. W., Wong C. P. W. (1976) J. Am. Chem. Soc. **98**: 7157
[2] Williams R. J. P. (1982) Struct. Bonding (Berlin) (1982) **50**: 79
[3] Bünzli J. C. G., Choppin G. R. (1989) Lanthanide Probes in Life, Chemical and Earth Sciences, Theory and Practice. Elsevier, Amsterdam
[4] Winkleman J., Slater G., Grossman J. (1967) J. Cancer Res. **27**: 2060
[5] Hambright P., Fawwaz R., Valk P., McRae J., Bearden A. J. (1975) Bioinorg. Chem. **5**: 87
[6] Marzola P., Cannistraro S. (1987) Physiol. Chem. Medical NMR **19**: 279
[7] Wei C. C., Hsu W. S., Tominaga Y., Tsasi J. C., Chai C. Y. (1993) Nuclear Instrum. and Meth. in Phys. Res. **B75**: 195
[8] Haye S., Hambright P. (1991) J. Coord. Chem. **22**: 315
[9] Lomova T. N., Andrianova L. G., Berezin B. D. (1988) Koord. Khim. **14**: 459
[10] Lavallee D. K. (1985) Coord. Chem. Rev. **61**: 55
[11] Mauzerall D. (1965) Biochem. **4**: 1801
[12] Hill H. A. O., Macfarlane A. J., Williams R. J. P. (1969) J. Chem. Soc. (A) 1704
[13] Krishnan V. (1984) Proc. Indian Acad. Sci. (Chem. Sci.) **93**: 767
[14] Pullman B., Pullmman A. (1964) In: Quantum Biochemistry, chapter IX. Interscience, New York
[15] Heathcote J. G., Hill G. J., Rothwell P., Slifkin M. A. (1968) Biochim. Biophys. Acta **153**: 13
[16] Sidorov A. N. (1974) Biofizyka **19**: 45
[17] Mehdi S. H., Brisbin D. A., McBryde W. A. E. (1975) J. Solution Chem. **4**: 497
[18] Horrocks W. D. W., Hove E. G. (1978) J. Am. Chem. Soc. **100**: 4386
[19] Wong C. P. (1982) Inorg. Synth. **22**: 156
[20] Radzki S., Krausz P., Giannotti C. (1987) Inorg. Chim. Acta. **138**: 139
[21] Suzuki N., Saitoch K., Shibata Y. (1990) J. Chromatogr. **504**: 179

[22] SigmaPlot. version 5.01. (1986–1982) Jandel Corporation
[23] Beck M. T. (1970) Chemistry of Complex Equilibria. Van Nostrand Reinhold Company, London, p. 93
[24] Sigma Plot. Scientific Graphic Software. User's Manual (1992) Jandel Scientific Corporation, Corte Madera, CA
[25] White W. J. (1978) In: Dolphin D (ed.) The porphyrins, vol. 5. Academic Press, New York, p. 303
[26] Burton G. W., Ingold K. U. (1986) Acc. Chem. Res. **19**: 194
[27] Foster R. (1969) Organic Charge Transfer Complexes. Academic Press, London
[28] Radzki S., Giannotti C. (1993) Inorg. Chim Acta **205**: 213

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