

Synthesis and Spectral Properties of Phthalocyanine–Methylpheophorbide *a* Covalently Linked Dyad

Irina O. Balashova,^a Victor E. Pushkarev,^{a@} Vladimir I. Shestov,^a Larisa G. Tomilova,^{a,b} Oscar I. Koifman,^c and Geli V. Ponomarev^d

^a*Institute of Physiologically Active Compounds, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation*

^b*Department of Chemistry, M.V. Lomonosov Moscow State University, 119991 Moscow, Russia*

^c*Research Institute of Macroheterocycles, Ivanovo State University of Chemistry and Technology, 153000 Ivanovo, Russian Federation*

^d*V.N. Orekhovich Institute of Biomedical Chemistry, 119121 Moscow, Russian Federation*

@Corresponding author E-mail: pushkarev@ipac.ac.ru

Phthalocyanine-methylpheophorbide a covalently linked conjugate was first synthesized via transesterification of the α -keto methyl ester in methylpheophorbide a with 2-(2-hydroxymethylbenzyloxy)-9(10),16(17),23(24)-tri-tert-butylphthalocyanine under mild conditions. UV-Vis absorption spectrum of the dyad exhibits panchromatic behavior owing to combination of the phthalocyanine and pheophorbide derived bands, while 1D and 2D NMR data indicate the presence of the intramolecular π -stacking interactions, and fluorescence emission spectrum in turn reflects energy transfer from the excited pheophorbide moiety to the phthalocyanine core.

Keywords: Phthalocyanine, methylpheophorbide *a*, transesterification, visible absorption, fluorescence.

Синтез и спектральные свойства ковалентно связанной диады фталоцианин–метилфеофорбид *a*

И. О. Балашова,^a В. Е. Пушкарев,^{a@} В. И. Шестов,^a Л. Г. Томилова,^{a,b} О. И. Койфман,^c Г. В. Пономарев^d

^a*Институт физиологически активных веществ РАН, 142432 Черноголовка, Россия*

^b*Московский государственный университет имени М.В. Ломоносова, Химический факультет, 119991 Москва, Россия*

^c*Ивановский государственный химико-технологический университет, 153000 Иваново, Россия*

^d*Институт биомедицинской химии имени В.Н. Ореховича, 119121 Москва, Россия*

@E-mail: pushkarev@ipac.ac.ru

*Впервые синтезирован ковалентный конъюгат состава фталоцианин-метилфеофорбид *a* путем переэтерификации α -кето метилового эфирного фрагмента метилфеофорбида *a* 2-(2-гидроксиметилбензилокси)-9(10),16(17),23(24)-три-трет-бутилфталоцианиновым лигандом в мягких условиях. Электронный спектр поглощения полученной диады демонстрирует панхроматическую природу благодаря комбинации полос феофорбидного и фталоцианинового хромофоров. Данные одномерной и двумерной ЯМР спектроскопии свидетельствуют о характерных π -стэкинговых взаимодействиях в молекуле диады, а исследование флуоресцентных свойств указывает на наличие внутримолекулярного переноса энергии от феофорбидного к фталоцианиновому фрагменту.*

Ключевые слова: Фталоцианин, метилфеофорбид *a*, переэтерификация, электронные спектры поглощения, флуоресценция.

Introduction

Molecular and supramolecular systems based on functional derivatives of tetrapyrrolic macroheterocycles in recent years cause an increased interest of researchers due to the possibility of a combination of certain useful properties. In particular, such systems are perspective for application in the field of theranostics – a modern direction in medicine combining therapy and diagnosis of diseases.^[1–3] Of special interest are covalent dyads representing the model system of chlorophyll special pair – a key component of a photosynthetic reaction center.^[4,5] Such systems typically involve porphyrin and/or pheophorbide *a* derivatives.^[6–9] More recent investigations also cover molecular systems combining porphyrin and phthalocyanine cores.^[10,11 and ref. therein] Note that the synthesis of unsymmetrically substituted phthalocyanine component is often characterized by low efficiency – the yield at this stage rarely exceeds 30 %. Our recent developments in the synthesis of low-symmetry A₃B type phthalocyanines allow to obtain these compounds in yields of about 50 % and make them available building blocks for the creation of covalent conjugates with various molecules and surfaces.^[12–17] The present communication is devoted to the synthesis and study of a novel type dyad based on phthalocyanine and methylpheophorbide *a*.

Experimental

UV-Vis and fluorescence spectra were recorded on Hitachi U-2900 spectrophotometer and Varian Cary Eclipse fluorescence spectrophotometer, respectively, in THF using quartz cells (10×10 mm). 1D and 2D ¹H NMR spectra were registered on Bruker AM-200 (200 MHz) and Bruker Avance 500 (500 MHz) instruments using CDCl₃ as the solvent. MALDI-TOF mass-spectra were obtained using Bruker Autoflex II mass spectrometer with α -cyano-4-hydroxycinnamic acid (CHCA) as the matrix. Gel permeation chromatography was performed on the polymeric basis Bio-Beads S-X1 (BIORAD) using toluene as the eluent. Preparative TLC was performed using Merck Silica Gel 60 flexible plates with toluene:MeOH (10:1 vol.) mixture as the eluent. Methylpheophorbide *a* (**1**) can be prepared by any of the known procedures.^[18–20]

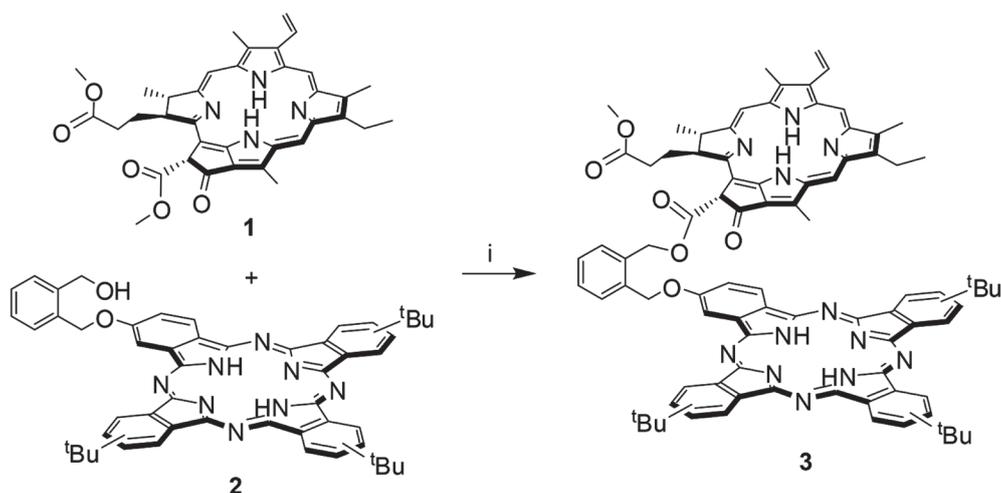
2-(2-hydroxymethylbenzyloxy)-9(10),16(17),23(24)-tri-*tert*-butylphthalocyanine (**2**) was synthesized following the published procedure.^[17] 4-Dimethylaminopyridine (DMAP) was purchased from Aldrich. All other reagents and solvents were obtained or distilled according to standard procedures. The reaction was TLC and UV/Vis controlled until complete disappearance of the starting phthalocyanine compound **2**.

Spectral data of the compounds 1 and 2. **1:** UV-Vis (THF) λ nm (lg ϵ): 277 (4.28), 321 (4.40), 410 (5.02), 506 (4.12), 535 (4.05), 609 (3.96), 667 (4.68). **2:** UV-Vis (THF) λ nm (lg ϵ): 288 (4.70), 340 (4.94), 601 (4.55), 642 (4.75), 661 (5.12), 697 (5.18).

Preparation of dyad 3. A mixture of methylpheophorbide *a* **1** (14 mg, 0.023 mmol), phthalocyanine **2** (12.6 mg, 0.015 mmol), DMAP (3 mg, 0.025 mmol) and catalytic amount of I₂ dissolved in 5 ml of toluene was refluxed for 3.5 h. The solvent was removed *in vacuo*, then the mixture was reprecipitated from the DMF:H₂O (99:1 vol.) mixture leading to partial separation of the unreacted pheophorbide **1**. Further purification was performed employing gel permeation chromatography, from which the second blue-green band was collected and dried *in vacuo* after evaporation of the solvent. The yield of **3** was 10.7 mg (51 %). Mass-spectrum (MALDI-TOF) *m/z*: 1393.68 [M+H]⁺, 698.30 [M–C₄₃H₄₃N₄O₅+H]⁺, 547.21 [M–C₅₂H₄₉N₈O₂–CH₃O+3H]⁺. ¹H NMR (CDCl₃) δ , ppm: 8.56–9.35 (m, 8H, α -H^{Ar,Pc}), 8.04–8.30 (m, 4H, β -H^{Ar,Pc}), 7.30–9.35 (group overlapped s, 3H, 5,10,20-H^{pheo}; m 1H, 3¹-H^{pheo}), 7.30–7.69 (m, 4H, H^{Ar,sp}), 5.85, 5.88 (2 s, 1H, 13²-H^{pheo}), 5.69–5.80 (m, 2H, 3²-H^{pheo}), 5.31–5.80 (m, 4H, H^{CH2,sp}), 3.84–3.98 (m, 1H, 18-H^{pheo}), 3.68–3.79 (m, 1H, 17-H^{pheo}), 3.53–3.61 (m, 3H, H^{MeO,pheo}), 3.18–3.25 (m, 2H, 8¹-H^{pheo}), 2.60–2.88 (group s, 3H, 7¹-H^{pheo}), 2.35–2.51 (m, 2H, 17¹-H^{pheo}), 2.16–2.36 (m, 2H, 17²-H^{pheo}), 1.48–1.98 (group s, 36H, H^{tBu,Pc} 2¹,12¹,18¹-H^{pheo}), 1.32–1.43 (m, 3H, 8²-H^{pheo}), -1.76, -2.23, -4.24, -4.54 (4 br s, 2H, N-H^{pheo}), -3.55, -3.75 (2 br s, 2H, N-H^{Pc}). UV-Vis (THF) λ nm (lg ϵ): 287 (4.85), 341 (5.11), 412 (5.04), 508 (4.09), 537 (4.08), 615sh (4.62), 667 (5.13), 702 (5.06).

Results and Discussion

The target dyad was obtained by transesterification reaction involving methylpheophorbide *a* **1** and functionally substituted phthalocyanine **2** with terminal OH-group of benzyl type (Scheme 1) following the modified conditions of the method earlier reported by Shinoda and co-authors^[6,7] for the transesterification of **1** by different alcohols including



Scheme 1. Synthesis of dyad **3**. *i* = I₂, DMAP, toluene, 110 °C.

porphyrin-based derivatives. Interaction of **1** and **2** was investigated both in the presence of a base (triethylamine, DMAP) and without the basic additives, while the optimal system found is shown in Scheme 1. It is shown that the use of chromatographic purification of the reaction mixture on the sorbents (SiO_2 , Al_2O_3) leads to a substantial reduction in the yield of the target dyad **3** apparently due to amplification of the oxidative processes. Therefore, we have developed the technique for isolation of the product, which excludes utilization of the sorption carriers and thus greatly simplifies scaling of the synthesis increasing the availability of the obtained dyad for the potential applied research.

The MALDI-TOF mass spectrum of conjugate **3** (Figure 1) along with the intense peak of the molecular ion $[M+H]^+$ contains weaker fragmentation peaks of the latter formed by cleavage of the ester bond of a spacer group. At the same time, the ^1H NMR spectrum of the dyad contains signals of both phthalocyanine and pheophorbide fragments (Figures 2 and 3). For comparison, ^1H NMR spectra of the initial ligands **1** and **2** are given in Figures 1S and 2S (in Supplementary Information), respectively. Note some shift of part of the signals in the spectrum of **3** to both stronger and weaker field relative to similar signals in the spectra of **1** and **2**, indicating mutual rapprochement of the phthalocyanine and pheophorbide macrocycles, which can be even enough for the somewhat π - π interactions. This assumption is in good agreement with the UV-Vis data (see below).

The UV-Vis absorption spectrum of dyad **3** (Figure 4) is close to a superposition of the spectra of its methylpheophorbide and phthalocyanine counterparts and resembles the spectrum of a stoichiometric mixture of the starting compounds **1** and **2** (Figures 5 and 6a,b). The spectral difference between **3** and the mixture of **1** and **2** lies in the ratio of the Q_{vib} (in 600–650 nm area) to the Q (at *ca.* 660 and 700 nm) band intensities implying the presence of the intramolecular π - π contact in **3**. At the same time, fluorescence spectra of dyad **3** and the equimolar mixture of **1** and **2** (Figure 6c,d) show a different intensity ratio for the bands corresponding to each of the components, indicating the presence of methylpheophorbide *a* to phthalocyanine intramolecular energy transfer in **3**.

Thus, certain interactions found in molecule of the first obtained phthalocyanine-methylpheophorbide *a* dyad together with exhibited optical properties show high prospectivity of this new class of compounds for further research and practical application as materials for theranostics.

Conclusions

First synthesis of covalent phthalocyanine-methylpheophorbide *a* dyad was carried out following a simple and effective transesterification of α -keto methyl ester group of methylpheophorbide *a*. Spectral properties of the resulting conjugate indicate the presence of intramolecular π - π interactions and evidence the possibility of the energy transfer from the methylpheophorbide *a* fragment to the phthalocyanine counterpart.

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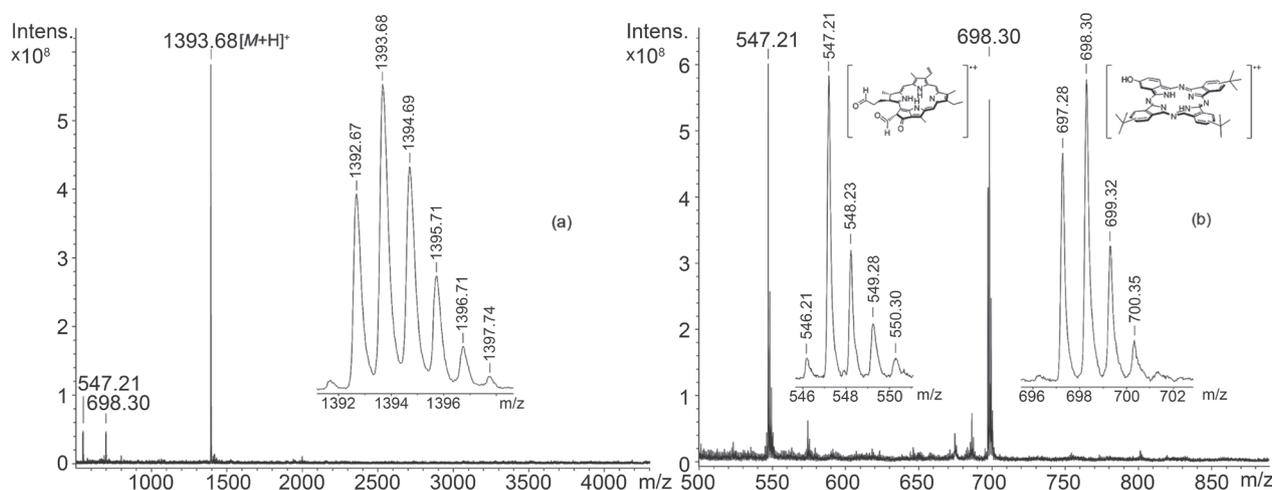


Figure 1. MALDI-TOF mass spectrum of dyad **3**: (a) full-range spectrum and (b) low-mass region of the spectrum showing fragmental ion peaks derived from $[M]^+$.

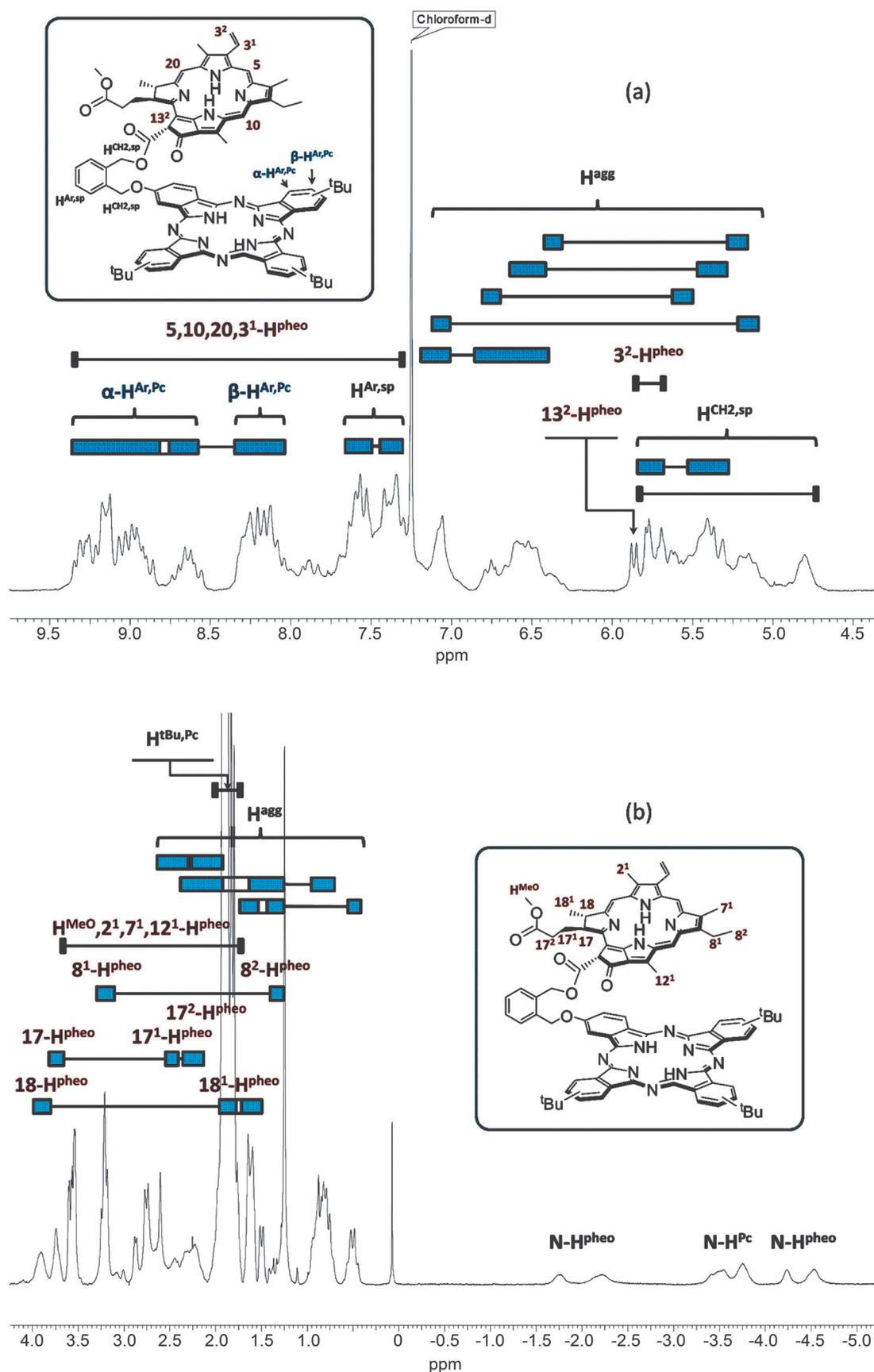


Figure 2. ^1H NMR spectrum of dyad **3** in CDCl_3 : (a) aromatic and (b) aliphatic region. Areas assigned to each type of the protons according to the 2D correlation are denoted by blue rectangles.

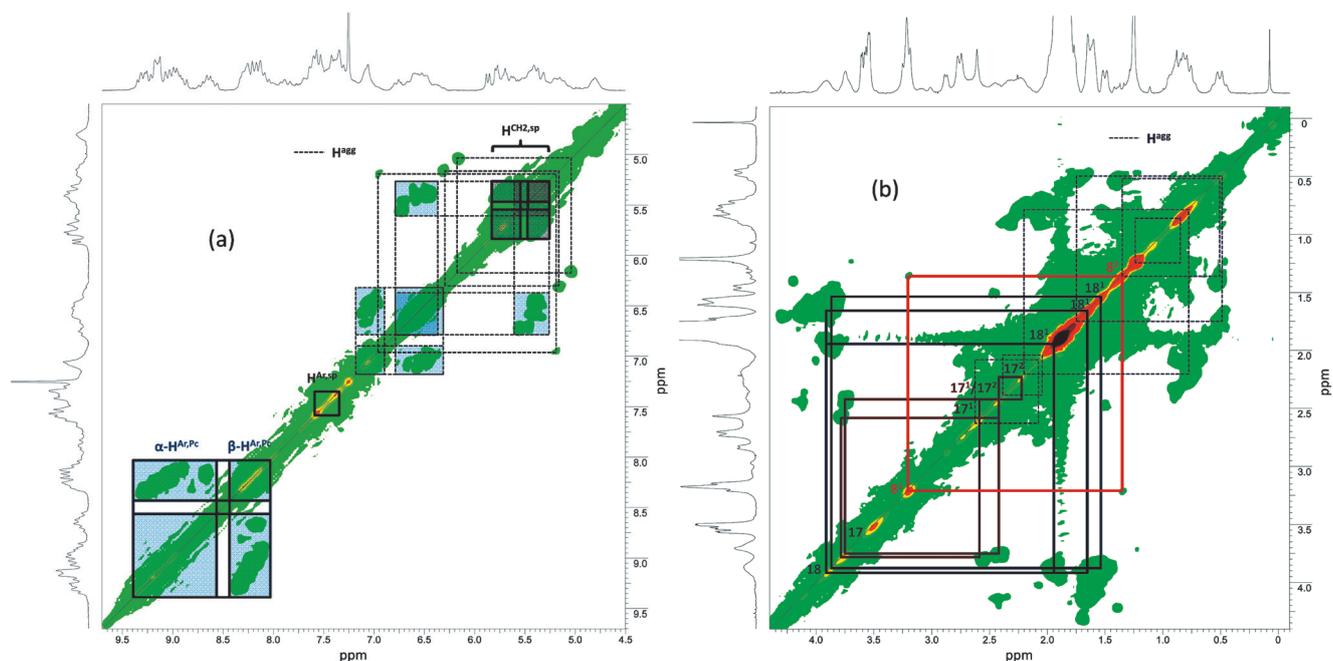


Figure 3. ^1H - ^1H COSY spectrum of dyad **3** in CDCl_3 : (a) aromatic and (b) aliphatic region.

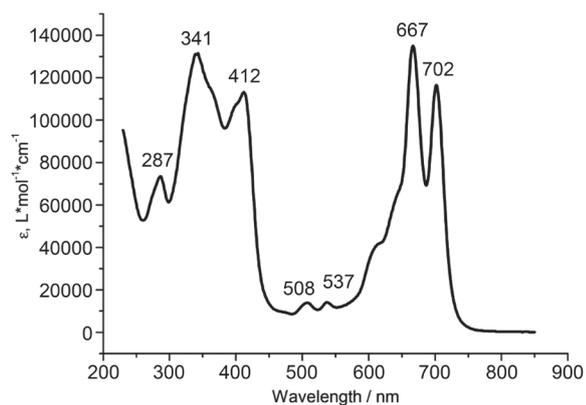


Figure 4. UV-vis spectrum of dyad **3** in THF.

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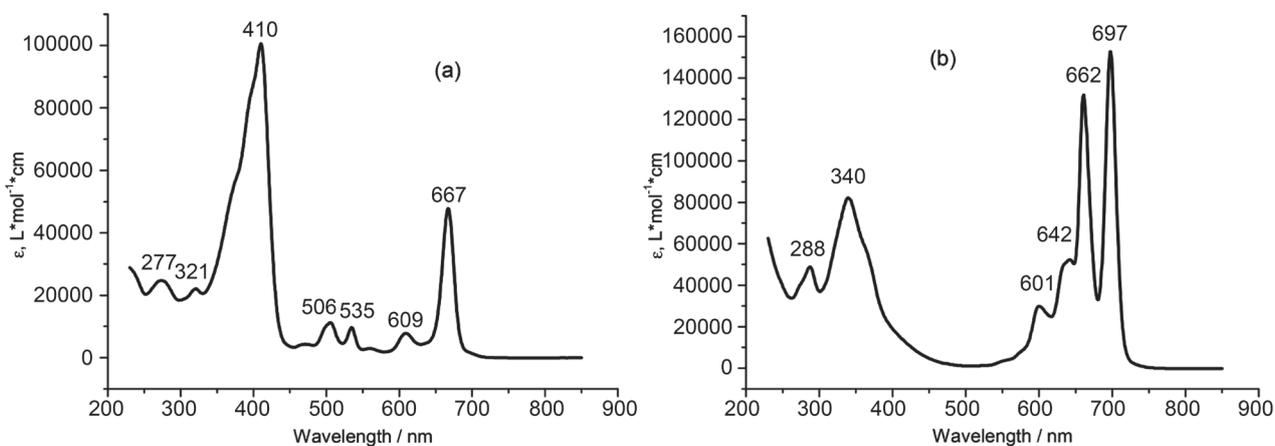


Figure 5. UV-vis spectra of methylpheophorbide **1** (a) and phthalocyanine **2** (b) in THF.

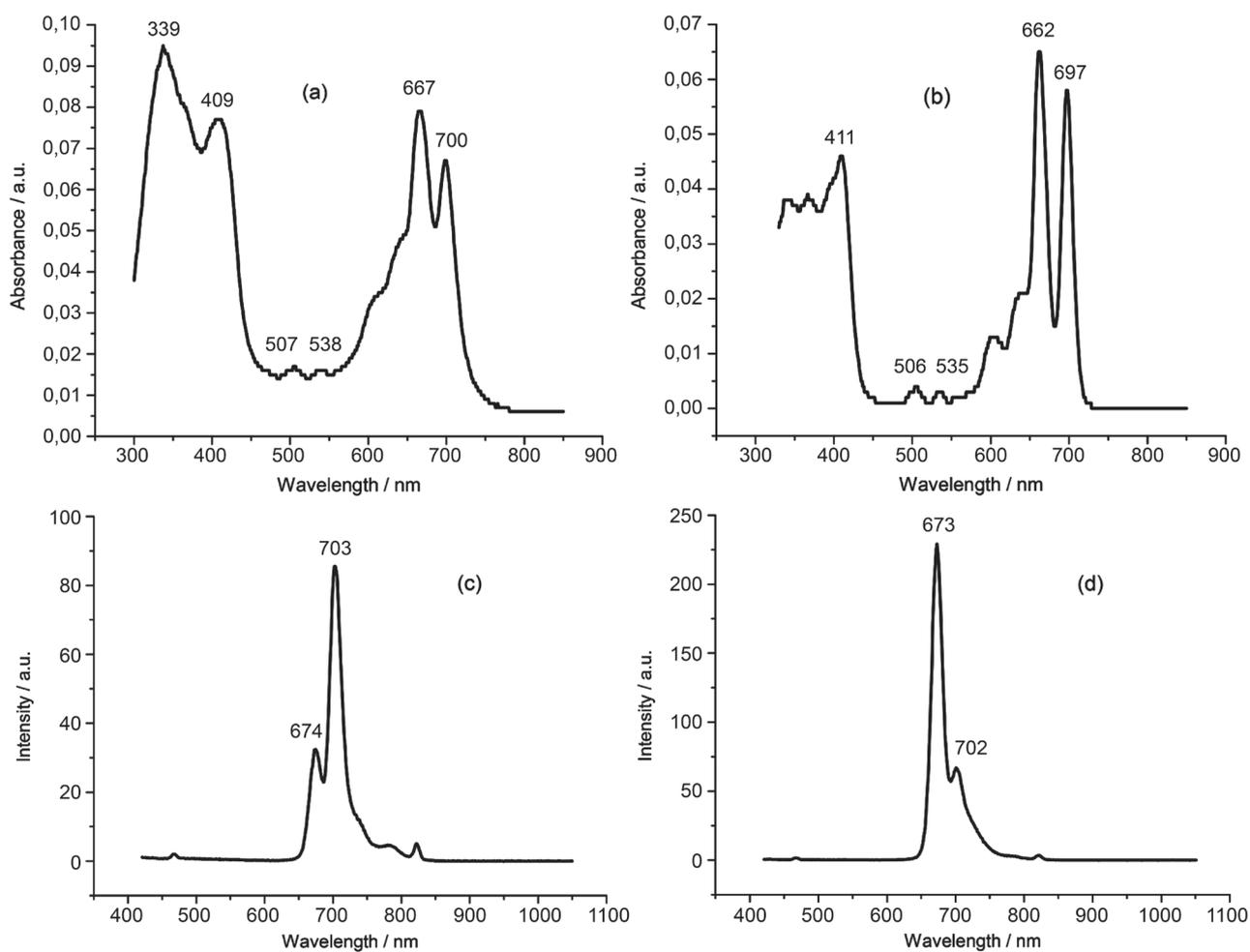


Figure 6. Fluorescence study of dyad **3** and stoichiometric mixture of **1** and **2** in THF. UV-vis spectra of the dyad (a) and the mixture (b). Fluorescence spectra of the dyad (c) and the mixture (d) ($\lambda_{\text{ex}} = 411$ nm).

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