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Synthesis of new binary porphyrin–cyanine conjugates and their self-aggregation in organic-aqueous media

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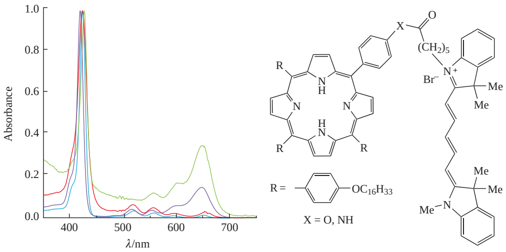
New amphiphilic conjugates of porphyrins and cyanine dye Cy5COOH were prepared by the creation of ester or amide bond between Cy5-carboxylic acid and hydroxy- or amino-substituted porphyrins. Study of self-aggregation of these conjugates in aqueous-organic media by dynamic light scattering and UV-VIS spectroscopy showed that the conjugates form micelles in THF–H_2O with different ratios.

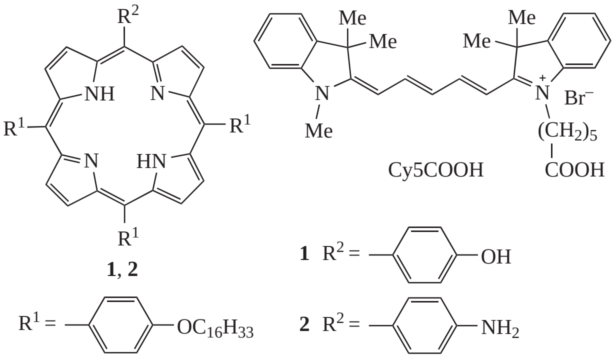


Photodynamic therapy is one of the promising approaches for the diagnosis and treatment of malignant neoplasms.[1,2] The method is based on the administration of a photosensitizer (PS) which upon light exposure produces disastrous reactive oxygen species. The therapy efficacy depends on many factors, *e.g.* chemical structure of used PS, its delivery method, cellular localization, *etc*.[3–6] Creation of binary systems combining the functions of the phototherapeutic and photodiagnostic agents (so called theranostics) is a new approach to obtain an effective PS.[7–9] Such systems include binary conjugates of tetrapyrrole compounds and dyes of the cyanine series. Nanoparticles containing mixture of cyanine dyes and porphyrins were reported.[10,11] A limited number of covalent conjugates are described. Thus, conjugates of 3-(1-hexyloxyethyl)-3-devinyl pyropheophorbide *a* (HPPH) and cyanine dye have been developed.[12–14]

Synthetic *meso*-arylporphyrins are increasingly used as agents for photodynamic therapy as model compounds due to their chemical availability and the convenience of the macrocycle periphery modification.[15,16] Lipophilic PSs are effective, but their use requires the development of delivery systems.[17] It is known that amphiphilic structure of porphyrins promotes self-assembly and facilitates their delivery and fusion into cell membrane.[18,19] Cyanine dyes have excellent photophysical properties (high extinction and quantum yield coefficients of fluorescence) and are widely used in biological detection, biovisualization, PCR diagnostics, and fluorescence *in situ* hybridization (FISH).[20–22] Self-assembly of supramolecular complexes of cyanine dyes is known.[23] However, they do not have specificity for tumor cells and are quickly eliminated from the body. Construction of a conjugate based on these dyes can avoid the drawbacks of individual components and provide new binary agents with desired physical and chemical properties.

In this paper, we propose binary amphiphilic conjugates based on porphyrins bearing higher alkyl substituents and cyanine dyes with useful photophysical characteristics for detecting and visualizing neoplasms (high extinction coefficients and quantum fluorescence yield). Two possible approaches to such hybrid systems were studied, which are based on the convenient preparative acylation of reactive hydroxyl or amino groups in the porphyrin precursors for the dye molecule attachment.

Previously, we showed efficiency of the monopyrrole condensation method in organic solvent mixture for the synthesis of non-symmetrically substituted porphyrins with active groups and high alkoxy substituents.[24–26] Porphyrins **1** and **2** were synthesized from the properly functionalized benzaldehydes.[†] Condensation of pyrrole, 4-(hexadecyloxy)benzaldehyde[27] and 4-hydroxybenzaldehyde/4-acetamidobenzaldehyde was conducted by their boiling in a mixture of nitrobenzene/propionic acid/acetic acid (1 : 2 : 1). Purification was carried out by column chromatography on silica gel G60 and recrystallization. Aminoporphyrin **2** was obtained after acidic hydrolytic removal of acyl group. The yields of the target precursors **1**, **2** were 10–12%. The Cy5COOH dye was prepared according to the reported method in 55% yield.[28] Indentity and structure of the starting compounds were confirmed by UV-VIS, ^{1}H, ^{13}C NMR spectroscopy and MALDI-TOF mass spectrometry.



1, **2**

Cy5COOH

$R^1 = -C_6H_4-OC_{16}H_{33}$

1 $R^2 = -C_6H_4-OH$

2 $R^2 = -C_6H_4-NH_2$

First approach suggests the formation of conjugate by creation of the ester bond between hydroxyporphyrin **1** and Cy5COOH. Acid Cy5COOH was treated with thionyl chloride (1.2 mol) in dichloromethane for 2 h, the reaction mass was evaporated, dissolved in dry THF and added to the solution of porphyrin and DMAP (1.5 equiv.) (Scheme 1, route 1). The ester product **3** was isolated by column chromatography in 10% yield.[†] Alternative

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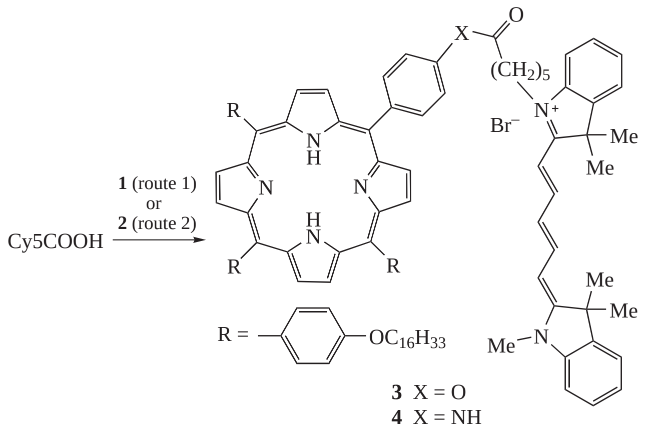
[†] For the syntheses and characteristics of compounds **1**–**4**, see Online Supplementary Materials.



3 X = O
4 X = NH

Scheme 1

strategy is based on the creation of amide bond between aminoporphyrin **2** and Cy5COOH in the presence of the condensing agent. First, we prepared activated *N*-hydroxysuccinimide (NHS) ester amine Cy5COOH according to known procedure.[29] To this NHS ester, amine **2** and calculated amounts of EDC [1-ethyl-3-(dimethylaminopropyl)carbodiimide] and DIPEA were added in anhydrous CH_2Cl_2 (see Scheme 1, route 2). The amide product **4** was isolated by column chromatography in 20% yield.[†] The second approach was preferable despite the longer (48 h) reaction time, as it provided higher yields of the conjugates. The structures of the conjugates **3** and **4** were confirmed by UV-VIS spectroscopy, ^{1}H NMR and MALDI-TOF mass spectrometry. The UV-VIS and fluorescence spectra of compounds **1**–**4** are shown in Figure 1. The UV-VIS spectra of conjugates contain Soret band and three bands in the region of 550–700 nm.

The resulting binary amphiphilic conjugates **3** and **4** are soluble both in organic solvents and in a water–alcohol solution. We suggested that substances with similar properties can self-assemble into micelle-like nanoparticles. Previously,[30] a simple way to obtain fluorescent organic nanoparticles, in which efficient fluorescence resonance energy transfer would generate singlet oxygen under linear and nonlinear light sources, was reported. We used similar approach to produce organic nanoparticles from conjugates **3** and **4**. Nanoparticles were obtained by injecting solutions of **3**, **4** into THF to reach concentration of 0.05 μM (the THF/H_2O ratio was 50 : 50 and 25 : 75, v/v), and the solutions were then left for a day in the refrigerator. The particle size was determined by dynamic light scattering (Table 1). With a THF/H_2O ratio of 50 : 50 for both compounds **3** and **4**, the formed particles had smaller dimensions compared to the ratio of 25 : 75. Also, these particles showed a narrower size distribution compared to published data,[30] which is a significant achievement of our work.

Probably, this was resulted from the presence of long-chain lateral substituents, which similarly to natural lipids are capable of self-organization into stable supramolecular associates. Also, the size of the nanoparticles is acceptable for their further use in photodynamic therapy of cancer tumors.

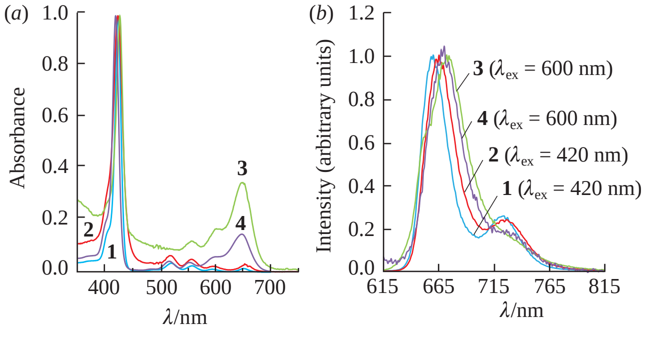


Figure 1 (*a*) Normalized UV-VIS absorption and (*b*) emission spectra of compounds **1**–**4** in THF.

Table 1 Particle size of compounds **3**, **4** obtained in THF–H_2O with different ratios.

Compound	THF : H_2O ratio	Particle size/nm
3	50 : 50	135.7 ± 9.9
	25 : 75	198.7 ± 77.3
4	50 : 50	78.25 ± 15.2
	25 : 75	465.3 ± 17.9

In conclusion, we have synthesized new conjugates based on porphyrins with long-chain alkoxy substituents and cyanine dye Cy5COOH. The conjugates are capable of self-organization into the fluorescent organic nanoparticles of acceptable dimensions for photodynamic therapy and have a narrow size distribution.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2018.11.021.

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