Synthesis by the Hummelen–Wudl Method and Physicochemical Study of Pyropheophorbide–Fullerene Dyad


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A new type of pyropheophorbide-fullerene[60] dyad was synthesized by their attachment by the cyclopropane backbone at the 13(1)-position of the chlorin macrocycle using the Hummelen-Wudl method. Here we report a complex analysis of the structure influence on the photophysical properties of the newly obtained dyad and its comparison with another pyropheophorbide-fullerene[60] dyad. The latter dyad was previously obtained by the attachment of the tetrahydro-pyrrole backbone at the third position of the macrocycle using the Prato reaction. It was shown by quantum-chemical modeling that the studied dyads have drastically different spatial positions of the fullerene core relative to the dye macrocycle plane: “parallel” for the former and “perpendicular” – for the latter. The influence of structural differences on various properties of dyads was studied: the aggregation properties, absorption spectra, the fluorescence quenching of the dye moiety in the dyads, and the lifetimes of excited states. The data obtained are important for the further development of methods for the directional design of the photoactive fullerene-dye structures – highly effective photosensitizers for photodynamic therapy.

Keywords: Pyropheophorbide, fullerene derivatives, dyad, synthesis, mass spectrometry, matrix-assisted ionization, fullerene-dye excimers relaxation kinetics, conformation.

Синтез методом Хюммелена–Вудла диады пирофеофорбид–фуллерен и исследование её физико–химических свойств

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Introduction

Hybrid structures based on dyes and fullerene \(^{C_{60}}\) attract researchers with their unique photophysical and electron-donor properties. They have already found wide applications in the fields of optoelectronics, photovoltaics, sensor design, and many other applications.\(^{1-5}\) Such fullerene-based structures are of particular interest as photosensitizers (PS) for photodynamic therapy (PDT). When the fullerene goes into the excited state, it can efficiently generate reactive oxygen species (ROS) and cause tumor damage. However, nowadays only a small share of the work on the fullerene-dye structures (so-called "dyads") is devoted to their use in PDT,\(^{6-8}\) and the urgent task is to find approaches that allow us to obtain the optimal combination of photophysical properties and high photodynamic activity of fullerene-dye dyads.

For the application in PDT, the promising way is the combination of fullerene with the dyes, which have high absorption in the red spectral region. Earlier, we have created a number of fullerene-chlorin dyads, for which a high generation efficiency of superoxide was shown.\(^{9-11}\) It was also shown that the decrease of linker length between the dye and fullerene core significantly increases the activity of the dyads.\(^{9,10}\) The second important point that should be taken into account is the fact that with each new addend attached to the fullerene core significantly decreases the ROS quantum yield (of both superoxide and singlet oxygen). This effect is caused by the disruption of \(^{C_{60}}\) spheroid symmetry, which leads to the increase of both LUMO energy and the energy of triplet excited state\(^{12-16}\) of fullerene.

Thus, the aim of the present work was to develop approaches to the synthesis of fullerene-chlorin dyads with a short linker and different mutual positions of the \(\pi\)-aromatic systems of the dye and the fullerene core and conduct a comparative analysis of the effect of the dyads structure on their photophysical properties. We selected two structures of fullerene-chlorin dyads that meet the above-listed requirements – the shortest linker length and the lowest possible degree of fullerene core modification. The already known fullerene-methylpyropheophorbide dyad\(^{19-21}\) obtained by the Prato reaction, was chosen as the first dyad, and the fullerene-pyropheophorbide dyad, which we obtained for the first time by the Hummelen-Wuud reaction, was chosen as the second dyad. In the present work, a comparative analysis of the influence of the structure of these dyads on their conformation, aggregation properties, absorption spectra, fluorescence, and lifetimes of excited states was carried out for the first time.

Experimental

The synthesis of dyad 1 and its precursors, methylpyropheophorbide \(a\) and \(d\) (Figure 1, compounds 2 and 3, respectively), as well as methods for its purification and characterization, are described in work.\(^{10}\) The synthesis of pyropheophorbide \(a\) (Figure 1, compound 4) was carried out according to the previously described procedure.\(^{22}\)

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tion of the product was carried out chromatographically on silica gel using toluene-isopropanol (1:1 vol.) as eluent. Product yield: 234 mg (49%). UV-Vis (CHCl3) λmax (relative absorption) nm: 669 (0.34), 610 (0.05), 507 (0.09), 410 (1.00). 1H NMR (500 MHz, CDCl3) δ ppm: 9.64 (1H, s-10), 9.54 (1H, s-5), 8.73 (1H, s-20), 8.18 (2H, d, J = 8.39 Hz, meta-Ts), 8.00 (1H, dd, J = 18.13 Hz, J = 11.61 Hz, 3(1)-CH), 7.43 (2H, d, J = 8.39 Hz, ortho-Ts), 6.32 (1H, d, J = 18.13 Hz, 3(2)-CH trans), 6.16 (1H, d, J = 11.61 Hz, 3(2)-CH cis), 5.33 (2H, dd, J = 19.08 Hz, 13(2)-CH), 4.59 (1H, m, 18(1)-CH), 3.65 (3H, s, 12(1)-CH 3), 3.37 (3H, s, 2(1)-CH 3), 2.24 (3H, s, 7(1)-CH 3), 2.62 (2H, m, 17(1)-CH 2), 2.44 (3H, s, CH 3 Ts), 2.24 (2H, m, 17(2)-CH 2), 1.81 (3H, d, J = 7.03 Hz, 18(2)-CH), 1.74 (t, J = 7.40 Hz, 8(2)-CH).

The synthesis of dyad II (Figure 1) was carried out by the Hummelen-Wudl reaction[23] under standard conditions. Tosylhydrazone pyropheophorbide 233.9 mg (0.33 mmol) and 19 m (0.35 mmol) of sodium methylene were dissolved in 5 mL of dry pyridine in an argon atmosphere. After that, a solution of 1.2 g (1.66 mmol) of fullerene C60 in 120 mL of dichlorobenzene was added into the reaction mixture. The obtained reaction mixture was stirred at the boiling temperature for 10 hours. After the reaction completion, the solvent was removed in vacuum, and the product of the reaction was dissolved in pyridine, and the solution was filtered from insoluble impurities. The filtrate was subjected to gel permeation chromatography using cross-linked polystyrene (Bio-Beads S-X1) as the stationary phase and pyridine as eluent. The supercontinuum probe signal out of the sample was dispersed by a polychromator (“Acton SP-300”, Roper Scientific, USA) and detected by CCD camera (“Newton”, Andor, USA). Transient absorption spectral changes ΔA(t, λ) were recorded within the range of 380–900 nm.

The spectra were corrected for group delay dispersion of the supercontinuum using the procedure described previously[26,27] and transferred through the skimmer to the mass analyzer. The relative polarizations of pump and probe beams were adjusted to 54.7° (the so-called “magic angle”).

The experiments were carried out at 293 K. The circulation rate in the flow cell was 8 mL/min. Pure dry argon was fed into the flow system to avoid contact of the sample solution with air. The supercontinuum probe signal out of the sample was dispersed by a polychromator (“Acton SP-300”, Roper Scientific, USA) and detected by CCD camera (“Newton”, Andor, USA). Transient absorption spectral changes ΔA(t, λ) were recorded within the range of 380–900 nm.

Particular attention was paid to the “coherence spike” or “coherent artifact”, which is observed at initial delays during the overlap of pump and probe pulses and complicates the analysis of the measured results at delays < 70 fs. The problem of the resonance signal of a chromophore molecule in the solution for the “coherent spike” time window was analyzed in accordance with work.[28]
the interaction with basic solvents and the formation of micellar nanoparticles.

It should be noted that for dyad II significantly stronger association processes were observed, which does not allow us to obtain the NMR spectrum for this dyad. The use of different solvents and their mixtures (benzene, carbon disulfide, chloroform, chloroform with the addition of trifluoroacetic acid, DMSO, pyridine with the addition of perfluoropyridine) also did not allow us to obtain the monomeric form of dyad II and register its NMR spectrum. Thus, such strong aggregation could not be explained only by hydrophobic interactions. We believe that in this case, exciton and dipole intermolecular interactions play a more significant role, determined by the specific spatial structure of dyad II, which will be discussed further.

Earlier, we reported that for dyad I, the MAI matrix-activated ionization method turned out to be the most effective,[29] which made it possible to record the mass spectra of the dyad itself and the product of double addition of the chlorine residue to fullerene, which could not be done by the APESI and MALDI methods due to fragmentation of their structure. A similar picture was found for dyad II: the application of the MALDI method did not give results due to the strong fragmentation of compounds under study, while the use of the MAI method allowed us to reliably record the mass spectrum of the compound (Figure 2A,B). It should be noted that in the case of dyad II, it was not protonated molecules of the dyad, but its sodium and potassium adducts (Na⁺, K⁺), that formed two peaks of molecular ions [M+Na]⁺ and [M+K]⁺ (Figure 2), with the practical absence of the peaks [M+H]⁺ and [M+2H]²⁺, characteristic mass spectra of the dyad I.[10] It can be assumed that this is due to the absence of pyrrolidinofullerene nitrogen atom in dyad II. Special studies are being carried out to explain the appearance in mass spectra of one or another ion-adduct of chemical compounds.[30] However, the results of these studies are more empirical.

The IR spectra of both pyropheophorbide-fullerene dyads under study had bands, characteristic of pyropheophorbide (about 1544, 1490, 840–680 cm⁻¹), and the fullerene core vibrations (bands 520, 606 and 1432 cm⁻¹). For dyad II with a free carboxyl group, an intense band was observed at 1682 and 1204 cm⁻¹, which correspond to this group.

Thus, despite the fact that it was not possible to register NMR spectra of dyad II, we think that the combination of applied purification methods and obtained mass-spectra, elemental analysis data, and IR spectra allow us unambiguously confirm the structure of dyad II.

Computational Simulation of Dyads Structures

Since the efficiency of electron and the excitation energy transfer processes between fragments of dyads strongly depends on the distance between these fragments, the spatial organization of the obtained dyads is of considerable interest. For the dyads under study, their 3D structure was calculated using the semi-empirical quantum-chemical method PM7[24] in the MOPAC 2016 software package.[35] The resulting models were presented in Figure 3; as can be seen from this figure, the conformations of dyad I and dyad II had significant differences. In dyad I, the dye fragment could be located almost parallel to the tangent plane of the fullerene sphere, which increased the efficiency of the interaction of the π-systems of the dye and fullerene core within the dyad.

On the contrary, for dyad II, which had a stiffer cyclopropane linker, the plane of the dye moiety was almost perpendicular to the tangent plane of the fullerene sphere.

Figure 1. The synthesis scheme of dyad I and dyad II. The synthesis of dyad I and its precursors are described in work.[10]
This configuration could significantly hamper the interaction of π-systems within one dyad, but might enhance intermolecular interactions – such configuration could be convenient for the formation of strong intermolecular π-stacking (Figure 3C), which, we believe, was the reason for the strong aggregation processes, observed for this dyad.

Absorption and Fluorescence Spectra

Upon the analysis of the photophysical properties of dyads, the compound methylpyropheophorbide a (MPP, Figure 1, compound 2) was also additionally analyzed as a comparison dye. The absorption spectra of dyad I coincide with those presented in work. For this dyad, a weak bathochromic shift of the Soret band (from 417 to 419 nm) and a hypsochromic shift (from 673 to 662 nm) for the Q-band peak compared to native dye MPP were observed (Figure 4A and Table 1). A similar picture was observed for analogous dyads from work.

In contrast to dyad I, the absorption spectrum of dyad II in the region of the Soret band was broader and had hypsochromic shift compared to the MPP (from 417 to 413 nm), while for the Q-band had the only band broadening without a noticeable peak shift (Figure 4A and Table 1). This effect could be caused by strong overlapping of π-orbitals of dye and fullerene systems due to the formation of dyad intermolecular aggregates in a pyridine solution, as it was discussed above.

Figure 2. Mass-spectra of dyad II: MAI (A) and MALDI (B).
Synthesis and Study of Pyropheophorbide-Fullerene Dyad

Table 1. Photophysical properties of fullerene-pyropheophorbide dyads (solvent – pyridine).

<table>
<thead>
<tr>
<th>Property \ Compound</th>
<th>MPP</th>
<th>dyad I</th>
<th>dyad II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance at Soret band peak ($\lambda_{Dmax}$), nm</td>
<td>417</td>
<td>419</td>
<td>413</td>
</tr>
<tr>
<td>Absorbance at Q-band peak ($\lambda_{Qmax}$), nm</td>
<td>673</td>
<td>662</td>
<td>673</td>
</tr>
<tr>
<td>Fluorescence peak ($\lambda_{FLmax}$), nm</td>
<td>676</td>
<td>–</td>
<td>670</td>
</tr>
<tr>
<td>Relative fluorescence quantum yield*</td>
<td>0.026</td>
<td>–</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fluorescence quenching compared to the free dye MPP, fold</td>
<td>1</td>
<td>&gt;5000</td>
<td>87</td>
</tr>
<tr>
<td>The calculated distance between the dye and the fullerene core centers, Å</td>
<td>–</td>
<td>6.54</td>
<td>10.2</td>
</tr>
<tr>
<td>Excited state lifetimes $\tau$, ps; and their fractional intensities ($A$, %)</td>
<td>–</td>
<td>$\tau_1 = 20$; ($A_1 = 100$)</td>
<td>$\tau_1 = 0.40 \pm 0.008$; ($A_1 = 61$)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\tau_2 = 12 \pm 0.3$; ($A_2 = 22$)</td>
<td>$\tau_3 = 100 \pm 1.8$; ($A_3 = 17$)</td>
</tr>
</tbody>
</table>

*It was calculated by the method\(^{[31]}\) using fluorescein as a reference compound.

Figure 4. Absorption spectra (A) and fluorescence (B) spectra: dyad I (1), dyad II (2), native dye MPP (3) and native fullerene C\(_{60}\) (4) (fluorescence not shown). All compounds were dissolved in pyridine at a concentration of 10 μM. For fluorescence $\lambda_{ex} = 417$ nm.

**Transient Absorption Measurements**

Time-resolved transition absorption spectroscopy was performed for both dyads with 670 nm excitation wavelength in pyridine solution. For dyad I, a reasonable standard deviation was obtained already by monoexponential approximation of the obtained kinetic data; more complex two- and three-exponential data approximations did not lead to a decrease in the standard deviation. The time constant of the relaxation process was ~20 ps (Figure 5A, and Table 1), which was close to the 26 ps value, previously reported for this dyad\(^{[19]}\) as the excimer state relaxation time. This relaxation pattern was in good agreement with the absence of a fluorescence signal for this dyad (Figure 6). More complex three- and four-exponential relaxation models, which were reported for the same dyad in earlier studies\(^{[19]}\) were probably associated with the presence of fluorescent impurities, the purification of which by sorption chromatography is practically impossible.\(^{[10]}\)

For dyad II, a more complex relaxation pattern was observed. The smallest standard deviation was achieved using a three-exponential model. The time constants were 0.40±0.008 ps, 12±0.3 ps, and 100±1.8 ps (Figure 5B,D, and Table 1). The process corresponding to a time constant of 0.4 ps can be attributed to the short-lived components of the fluorescence decay, or ultrafast internal conversion (Figure 6). Approximately 61 % of the excited molecules relax by this pathway. A time constant of 12 ps correlates well with intermolecular relaxation processes; this relaxation path brings another 22 % of the excited molecules to the ground state. Such large value also indicates strong association processes of dyad II in pyridine solution. The time constant of 100 ps could be attributed to the relaxation process of the excimer state; the weight contribution of this process was about 17 %.

The slower excimers relaxation kinetics for dyad II, compared to dyad I, as well as the lower efficiency of its formation, could be explained by their different spatial conformation. Dyad I had an optimal configuration.
Figure 5. Time-resolved transient absorption curves of dyads: dyad I (A) and dyad II (B). The solid lines correspond to the global fittings. The initial transient absorption spectra of dyads are also shown: dyad I (C) and dyad II (D). Dyads were dissolved in pyridine; all spectra of samples were recorded after excitation by a 25-fs laser pulse at 670 nm.

Figure 6. Transient states and possible excited state relaxation pathways of dyads under study.
for electron transfer between dye and fullerene π-systems and a minimum distance between the molecule, which leads to the effective formation of fullerene-dye excimers, and also to rapid relaxation of this state. For dyad II, a change in the relative location of dye and fullerene moieties led to an increase in the distance between dyad fragments (Table 1) and to a decrease in the electron transfer efficiency between these fragments. It was manifested in the appearance of a fluorescence signal for dyad II, in the decrease of excimer relaxation rate and, consequently, in the significant decrease of the electron transfer process contribution to the excited state relaxation of the dye moiety of the dyad.

A similar picture of the excited states lifetimes is described for analogous fullerene-porphyrin and fullerene-chlorin systems, and the obtained excimer state lifetimes for the studied dyads (20–100 ps) turned out to be one of the shortest for such systems.[20,32] Apparently, this is due both to the short linker length between the fullerene core and the dye, and, in the case of dyad I, also to the orientation of the dye relative to the fullerene core that is optimal for excimer formation. Another factor that could further reduce the of the dyad II lifetime – is the type of solvent (pyridine); solvents with relatively low polarity could hamper the formation of excimer or charge transfer states.

**Conclusion**

Thus, we developed a new approach to the synthesis of the fullerene-pyropheophorbide dyad based on the Hummelen-Wudl reaction (dyad II). For this dyad and the previously obtained dyad I, a comparative analysis of the influence of the mutual arrangement of the π-aromatic systems of the dye and the fullerene core on the physicochemical properties of the studied dyads was carried out. Dyad I had a “parallel” spatial conformation that ensures the predominance of the intramolecular interaction of the dye and fullerene moieties. This dyad was characterized by the dominant decay path of the excited state through the excimer state. On the contrary, dyad II “perpendicular” spatial conformation enhanced the intermolecular interaction, and the decay of the excited state through excimers was significantly suppressed by other relaxation paths.

Thus, upon the change of the location of the dye and fullerene moieties relative to each other, due to the various conformations, it was possible to significantly affect both the efficiency of the interaction of dye and fullerene moieties and the aggregation properties of dyads, which, in turn, determined the nature and relaxation times of dyad excited states.

The obtained data are important for the further development of directional design methods of photoactive fullerene-dye dyads as new highly effective photosensitizers for photodynamic therapy.

**Acknowledgements.** The transient absorption measurements of dyads were performed using the facilities of Semenov FRCCP RAS CCE (N 506694). The synthesis of dyads and the analysis of their photophysical properties were funded by the Russian Science Foundation (project N 18-74-00128). The analysis of dyads by MAI method was supported by the Program of Basic Researches for Russian State Academies of Sciences 2013-2020 (state registration NAAAA-A18-118112690060-9).

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**Макрогетероциклы / Macrocycles** 2020 13(2) 147-155
A. Yu. Rybkin et al.


Received 04.05.2020
Accepted 09.06.2020