Pyropheophorbide–Fullerene Dyad: Synthesis and Photochemical Properties

Alexander Yu. Rybkin,a,© Alexandra Yu. Belik,a Pavel A. Tarakanov,a,c Kamil R. Taziev,b,a Alexei V. Kozlov,a Nikolay S. Goryachev,a,b Ilya V. Sulimenkov,d Viatcheslav I. Kozlovskiy,d,c Yulia V. Romanenko,e Oscar I. Koifman,e and Alexander I. Kotelnikova,b

*a Institute of Problems of Chemical Physics RAS, 142432 Chernogolovka, Russia
b Lomonosov Moscow State University, 119991 Moscow, Russia
c Institute of Physiologically Active Compounds RAS, 142432 Chernogolovka, Russia
d Talrose Institute for Energy Problems of Chemical Physics (Branch) RAS, 142432 Chernogolovka, Russia
e Ivanovo State University of Chemistry and Technology, 153000 Ivanovo, Russia
© Corresponding author e-mail: alryb@icp.ac.ru

The methylpyropheophorbide-fullerene[60] dyad was synthesized by 1,3-dipolar cycloadditions of the corresponding azomethine ylide to C_{60} (Prato reaction). Using the mass spectrometric method with soft matrix-activated ionization it was possible to achieve a significant reduction in fragmentation processes by the retro-Diels-Alder reaction, which allows to reliably detect the presence of polyadducts of azomethine ylide cycloadditions to fullerene. The use of gel permeation chromatography under conditions of weakening of the intermolecular π-π interaction between methylpyropheophorbide and fullerene moieties makes it possible to effectively separate mixed products with ~ 1.5 fold difference in molecular weight. It has been shown that the fluorescence of the dyad is quenched more than 5000 times (compared to the native dye). The singlet oxygen quantum yield of the dyad is 360 times less than that for the native methylpyropheophorbide a, however, its efficiency of superoxide generation increases by 18.5 times. The obtained result agrees well with the previously reported mechanism of relaxation of the excited state of the dyad through a charge-separated state, which can lead to the formation of superoxide. The observed effects indicate a change in the mechanism of photodynamic activity from type II (generation of singlet oxygen) for the native dye to type I (generation of superoxide) for the dyad, which shows a promising method of creation of highly efficient photosensitizers based on similar dye-fullerene[60] dyads.

Keywords: Pyropheophorbide, fullerene derivatives, fluorescence quantum yield, photochemical activity, singlet oxygen, mass spectrometry, matrix-assisted ionization.
Pyropheophorbide-Fullerene Dyad

Introduction

Currently, active work is underway to create photosensitive structures based on fullerene-dye dyads for photovoltaics and a number of other applications. These dyads are of particular interest for photodynamic therapy as photosensitizers, since fullerene C₆₀ has extremely high photoactivity — under light excitation it is capable of passing to an excited triplet state with almost 100 % quantum yield and effectively generate reactive oxygen species (ROS).

According to the mechanism of photodynamic action, photosensitizers are divided into two main categories: type I (generation of superoxide by the mechanism FS⁺ + O₂ = FS+ + O₂⁻) and type II (generation of singlet oxygen by the mechanism FS⁺ + O₂ = FS + O₂). The vast majority of photosensitizers that are used in clinical practice generate ROS according to type II mechanism. The creation of highly efficient photosensitizers that generate ROS through both paths I and II is of considerable interest since it is expected that they will be used in photovoltaics and the efficiency ROS generation was not evaluated. In the present paper, the ability of the pyropheophorbide-fullerene dyad to generate ROS through I and II pathways is compared for the first time.

Experimental

Synthesis of pyropheophorbide a is carried out according to the previously described method. Product yield: 86.4 %. MS (ESI) m/z 548 [M⁺], calcd for C₃₄H₃₆N₄O₃ 548. UV-Vis (C₆H₅CN) λ (relative absorption) nm: 669 (0.42), 611 (0.05), 539 (0.05), 509 (0.07), 415 (1.00). ¹H NMR (200 MHz, CDCl₃) δ ppm: 9.43 (1H, s, 10-CH), 9.31 (1H, s, 5-CH), 8.48 (1H, d, J = 20.2 Hz, 17-1)-CH₃), 1.62 (t, J = 7.2 Hz, 18(1)-CH₃), 1.74 (3H, q, J = 7.6 Hz, 8(2)-CH₃), –1.76 (1.4H, s, 21,23-NH).

Synthesis of methylpyropheophorbide b is carried out according to the previously described method. The product was chromatographed on silica gel, eluent 0.5 % methanol solution in methylene chloride. Product yield: 61.7 %. MS (ESI) m/z 551 [M⁺], calcd for C₃₅H₃₆N₄O₅ 551. UV-Vis (CH₂Cl₂) λ (relative absorption) nm: 697.8 (0.75), 637.3 (0.09), 557.7 (0.16), 523.8 (0.14), 429.4 (1.00), 389.2 (0.85). ¹H NMR (200 MHz, CDCl₃) δ ppm: 182

Макрогетероциклы / Macroheterocycles 2019 12(2) 181-186
11.41 (1H, s, 3(1)-CH), 10.12 (1H, s,10-CH), 9.43 (1H, s, 5-CH), 8.75 (1H, s, 20-CH), 5.18 (2H, q, J = 20.3 Hz, 13-CH), 4.49 (1H, m, 18-CH), 4.30 (1H, m, 17-CH), 3.67 (3H, s, 2(1)-CH3), 3.57 (8H, m, 12(1)-CH3), 8(1)-CH2, 17(3)-OCH3), 3.16 (3H, s, 7(1)-CH3), 2.59 (2H, m, 17(1)-CH3), 2.24 (2H, m, 17(2)-CH2), 1.78 (3H, d, J = 7.2 Hz, 18(1)-CH), 1.59 (3H, t, J = 7.6 Hz, 8(2)-CH2), –0.36 (0.5H, brs, 21-NH), –2.26 (0.7H, brs, 23-NH).

Synthesis of dyad fullerene-methylpyropheophorbide by Prato reaction was carried out according to the previously described method with some changes. Methylpyropheophorbide d (0.167 g, 0.3 mM), fullerene C60 (1.506 g, 2.09 mM) and sarcosine (0.135 g, 1.52 mM) were dissolved in 70 ml of o-dichlorobenzene. The resulting solution was subjected to ultrasonic irradiation then refluxed and stirring under an inert atmosphere for 24 hours. After completion of the reaction, the solvent was removed under vacuum, the dry residue was dissolved in 500 ml of a 30 % solution of trifluoroacetic acid in chloroform and filtered through a glass filter. Next, the filtered solution was evaporated, the resulting dry residue was dissolved in pyridine. Pyridine solution of the reaction mass (1 mg/ml) was subjected to gel permeation chromatography using cross-linked polystyrene (Bio-Beads S-X1) as the stationary phase. Product yield: 0.132 mg (33 %). 1H NMR (500 MHz, CDCl3) δ ppm: 11.72, 9.94 (1H, br.m, 5-CH), 9.78 (1H, br.m, 10-CH), 8.91, 8.85 (1H, brs, 10-CH), 6.81, 6.37 (1H, brs, 2-CH), 5.41–5.03 (3H, m, 5'-CH, 12(2)-CH2), 4.62–4.49 (2H, m, 5'-CH, 18-CH), 4.39–4.28 (1H, m, 17-CH), 3.74–3.38 (14H, m, 2(1)-CH3, 8(1)-CH2, 17(2)-CH2, 17(3)-OCH3, 7(1)-CH3), 2.75–2.17 (4H, m, 17(1)-CH3, 17(2)-CH2), 1.88–1.79 (3H, m, 18(2)-CH2), 1.69–1.55 (3H, m, 8(2)-CH2), –1.95, –2.07 (1.5H, s, 21,23-NH). High resolution MS (HR MS) m/z 1298.3143 [M+H]+, calcd for C95H40N5O3 1298.3126.

Matrix Activated Ionization Method (MAI)

Mass spectra were acquired using the Exactive Orbitrap high resolution mass spectrometer (ThermoFisher Scientific, Germany). The sample solutions in the concentrated trifluoroacetic acid were mixed with a saturated solution of the matrix (Phthalonitrile) in acetonitrile in 1:1 ratio. The concentration of the sample in the prepared mixture was deposited to the thin metal wire. After the short drying at the ambient temperature the matrix activated ionization method (MAI) was performed. Product yield: 0.132 mg (33 %). 1H NMR (500 MHz, CDCl3) δ ppm: 11.72, 9.94 (1H, br.m, 5-CH), 9.78 (1H, br.m, 10-CH), 8.91, 8.85 (1H, brs, 10-CH), 6.81, 6.37 (1H, brs, 2-CH), 5.41–5.03 (3H, m, 5'-CH, 12(2)-CH2), 4.62–4.49 (2H, m, 5'-CH, 18-CH), 4.39–4.28 (1H, m, 17-CH), 3.74–3.38 (14H, m, 2(1)-CH3, 8(1)-CH2, 17(2)-CH2, 17(3)-OCH3, 7(1)-CH3), 2.75–2.17 (4H, m, 17(1)-CH3, 17(2)-CH2), 1.88–1.79 (3H, m, 18(2)-CH2), 1.69–1.55 (3H, m, 8(2)-CH2), –1.95, –2.07 (1.5H, s, 21,23-NH). High resolution MS (HR MS) m/z 1298.3143 [M+H]+, calcd for C95H40N5O3 1298.3126. UV-Vis (ε, H2O) λ (lg ε) nm: 662 (4.63), 506 (4.37), 459 (5.11).

Separation and Characterization of Reaction Products

1,3-Dipolar cycloaddition reaction of azomethinylides to the fullerene core proceeds with low selectivity and leads to the formation of side polyadducts. The use of column liquid chromatography on silica gel to separate adducts of mono- and di-addition of azomethinylide derivatives of pyropheophorbide a to fullerene, described in,[24] proved ineffective. Only the thin-layer chromatography conditions used in this work could allow separating the resulting mixture 2 and 3. However, it was not possible to use this method for quantitative separation due to the close relative mobility of the components (2 and 3) transfer over the TLC plate. Gel permeation chromatography allows one to separate dyads from polyadducts products with high efficiency.[24] However, due to the effective intermolecular π-π donor-acceptor interaction between the porphyrin-fullerene entities,[29] in our case, additional restrictions are imposed on the maximum concentration in the final reaction mass of the conjugates 2, 3, as well as the content of the methylpyropheophorbide d and fullerene and their side products, to prevent the formation of supramolecular aggregates of mixed composition under gel permeation chromatography. Thus, a sevenfold excess of fullerene and an increase in the reaction temperature led to a significant decrease of the starting methylpyropheophorbide d and its side products in the final reaction mass. Dissolving the reaction mass in a 30 % solution trifluoroacetic acid in chloroform followed by filtering allows to quantitatively separating from fullerene. Moreover, a dry product, evaporated on a rotary evaporator after contact with acid has better solubility in organic solvents compared to the one before the contact. The observed effect is most likely associated with the destruction of intermolecular interactions between the chlorin-fullerene entities – as a result of the protonation of the nitrogen atom as a tertiary amine, as well as intracyclic nitrogen atoms of chlorin macroheterocycle. Thus, gel permeation chromatography in pyridine leads to a quantitative separation of the reaction mass (with the concentration of the reaction mass of 1 mg/ml) into three main fractions: triad 3, dyad 2 and unsubstituted fullerene with traces of chlorin. Unfortunately, a change in the synthesis conditions did not lead to an increase in the yield of the target monoaaddition product 2, however, reducing the content of chlorin structures in the reaction products made it possible to avoid additional column liquid chromatography on silica gel. Thus, reaction mass obtained by the method[24] in similar conditions does not allow to get adequate purity of the product 2 using gel permeation chromatography.
In this case, even additional column liquid chromatography on silica gel leads to unpurified product 2, containing both methylpyropheophorbide d itself and its chlorin derivatives side products, as evidenced by fluorescence and excitation spectra (Figure S6, 7).

As it can be seen from Figure 1, the main bi-product is the product 3; however, this compound was not detected using the MALDI ion source for mass-spectral analysis. This may be due to the inverse reaction (retro Diels-Alder), since such a reaction can be activated by an intensive ultraviolet radiation pulse used in the MALDI ion source. Using an electrospray ion source for mass-spectral analysis of samples is complicated by the poor solubility of our compounds. The samples were dissolved in the concentrated trifluoroacetic acid. Using an electrospray ion source these solutions must be diluted with a suitable solvent, such as acetonitrile or methanol, since a concentrated acid injection to an electrospray emitter can destroy the ion source. But under the dilution, the concentration of the dissolved sample decreases, and the sample can precipitate in the resulting diluted solution. Several years ago, an alternative ionization method was proposed – the so called matrix-activated ionization of samples under study [30] (MAI). Using a modification of this method it was possible to reliably detect products 2 and 3. The mass spectra of the solutions from two chromatographic fractions are presented in Figure 1. Analysis of the isotopic distributions of the detected ion peaks and accurate measurement of the ion \( m/z \) values allowed to reliably identify the main registered ions. In the mass spectrum in Figure 1A one can see the intense peaks of protonated (singly and doubly charged) product 2 ions. In this mass spectrum there are no any “traces” of protonated product 3 ions. On the contrary, in the mass spectrum in Figure 1B, there are the intense protonated (single, double and triple charged) product 3 ions. The intensity of the singly charged product 2 ions in this mass spectrum is about 12 % of the maximal intensity.

**Absorption and Fluorescence Spectra**

As can be seen from the absorption spectra (Figure 2A), a weak bathochromic shift of the Soret band is observed (from 417 to 419 nm), which is typical for fullerene-dye dyads, however, for the peak in the Q-band region, the opposite hypsochromic shift is observed (from 673 to 662 nm). The same picture is observed for similar dyads from paper. As we can observe in Figure 2B, the fluorescence of the dyad is completely quenched (more than 5000 times), the sensitivity of the device used does not allow it to be reliably detected (Figure 2B, in the inset) and calculate the fluorescence quantum yield. For similar dyads, which were previously presented, strong quenching of fluorescence was also reported (50–200 times), however, such a strong quenching for this dyad was observed for the first time. The residual fluorescence is probably associated with a small fraction of the impurity dye, which was not fully separated by the previously used purification methods, as it was already discussed above (Figures S6 and S7).

The high efficiency of fluorescence quenching of the dyad could be explained by the effective electron transfer from the excited state of the dye to the fullerene core. For a similar dyad, the formation of a charge-transfer state by flash photolysis was shown, which confirms the possibility of fluorescence quenching by the electron transfer mechanism.

On the contrary, the probability of fluorescence quenching by the Forster energy transfer mechanism is extremely small, as in the case of the fullerene-chlorin dyad described in our previous work, since the absorption of fullerene in the region of fluorescence of the dye is negligible.

As it can be seen from Figure 3A, combining dye and fullerene in one dyad leads to a significant reduction of singlet oxygen generation efficiency (360 times) compared with the native dye methylpyropheophorbide a.
The observed effect could be associated with the competitive way of fluorescence quenching not through the excited triplet state, but as a result of electron transfer and the formation of a long-lived charge-separation state, which was shown for an identical dyad in the papers,[19,20] which could lead to a decreases of the quantum yield of singlet oxygen. At the same time, the superoxide generation efficiency of the dyad increases by 18.5 times compared with the native dye (Figure 3B). The obtained result is also in a good agreement with the assumption about the formation of a long-lived charge-separated state in dyad 2, which can lead to the formation of superoxide.

Thus, the observed effects indicate a change of the photodynamic activity mechanism from a type II reaction (singlet oxygen generation) for the original dye methylpyropheophorbide \( \alpha \) to the type I reaction (superoxide generation) for dyad 2.

In our next work we are going to study the electron transfer process in such structures, perform the optimization of a number of parameters (linker length between dye and fullerene, redox potentials of the dye and fullerene derivative), which will open the way for the creation of new highly efficient photosensitizers for photodynamic therapy.

The further development of the synthesis, separation and detection methods of such fullerene-dye dyads in order to obtain high purity compounds is also of key importance for the study of their biological activity and their medical applications.

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Pyropheophorbide-Fullerene Dyad

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