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Synthesis and photophysical properties of heptamethine cyanine–fullerene C_{60} dyads with non-quenched fluorescence

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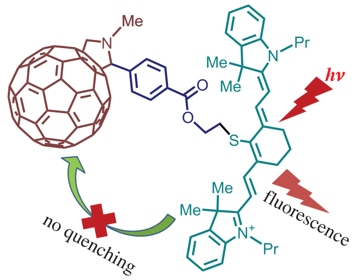
Two new heptamethine cyanine dye–fullerene C_{60} covalently-linked dyads, which absorb in far-red and NIR spectral regions, have been synthesized by esterification click reaction and characterized by physicochemical methods. No significant fluorescence quenching was found due to weak electronic coupling between heptamethine moiety and fullerene core, which was confirmed by photophysical and electrochemical methods. Such dyads can be useful for cell imaging and fluorescence diagnostics of various fullerene derivatives.

Fullerene C_{60}–heptamethine cyanine dye dyads

✓ high NIR absorbance
✓ no fluorescence quenching

perspective tool for cell imaging and fluorescence diagnostics of fullerene derivatives



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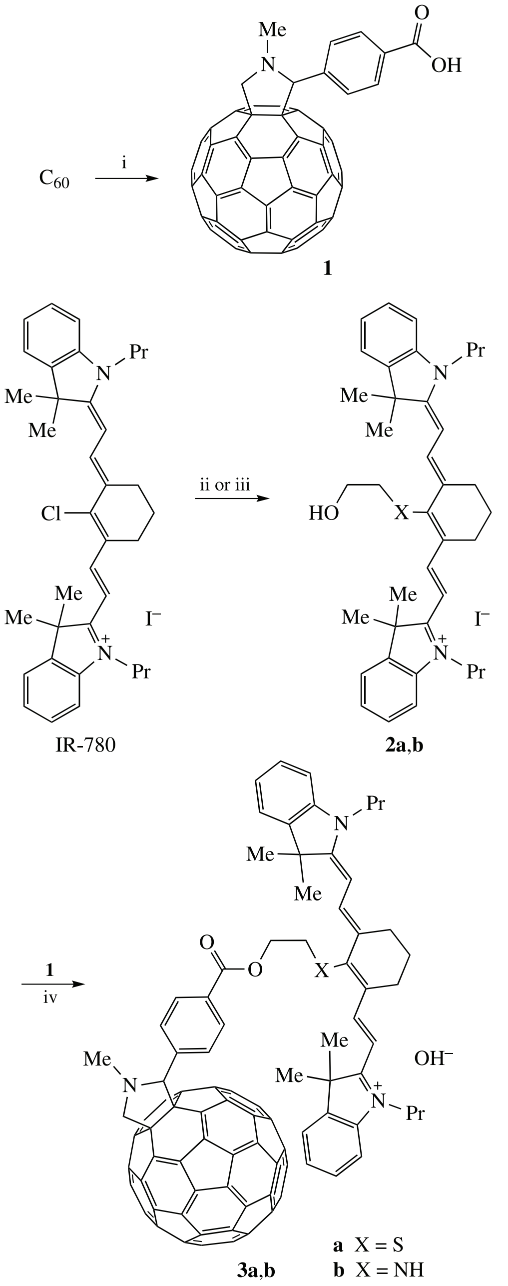
Fullerene–dye structures (also referred to as ‘dyads’) possess unique features, the most important among them being capable of forming long-lived charge-separated states upon photoexcitation successfully applied in photovoltaics.[1] Another feature is the ability of fullerene to act as a ‘spin converter’ and to form the excited triplet state upon interaction with a dye in an excited singlet state.[2] This ability is highly desirable for the design of the so-called ‘heavy-atom-free’ triplet photosensitizers.[2,3] Additionally, these two features are anticipated to drastically expand the range of potential drugs for photodynamic therapy since almost any dye is suitable for creation of type I or type II photosensitizers.[3,4] However, only a few works deal with fullerene–dye dyads with pronounced absorption in the near-infrared (NIR) region of the spectrum.[5,6] This region is highly attractive for photodynamic therapy and fluorescence diagnostics because living tissues have the most pronounced transparency in this region.

This work is devoted to the synthesis and analysis of two (fullerene C_{60})–heptamethine cyanine dye dyads which exhibit pronounced absorption in the red or NIR regions of the spectrum. The synthesis is outlined in Scheme 1 (for experimental details and full characterization, see Online Supplementary Materials). It is of note that the ‘click-chemistry’ esterification can be employed to attach almost any cyanine dye to any fullerene derivative bearing carboxy group. In our hands, the yields of products **3a** and **3b** amounted to 60 and 58%, respectively, compared to the previously reported cases (30–40%) for analogous fullerene–cyanine dyads.[5,6] Electrospray ionization mass spectra of compounds **3a**,**b** contained true molecular ion peaks (see Online Supplementary Materials, Figure S2), however these dyads turned unstable under conditions of the ESI measurements and formed a large number of fragments. The similar phenomenon was observed previously[7] for the fullerene–pyropheophorbide dyads.

The absorption spectrum of compound **2a** is almost identical to that of precursor IR-780 cyanine dye; however, its extinction coefficient decreases by a factor of 2.6 (Figure 1, Table 1). In contrast to **2a**, the absorption maximum of **3b** has a pronounced hypsochromic shift (λ_{max} 803 and 654 nm, respectively), which is in good agreement with the reported data.[8]

When these dyes are attached to the fullerene derivative **1**, a hypsochromic shift of their absorption peaks is observed; dyad **3a** has the most pronounced shift (from 802 to 753 nm, see Figure 1 and Table 1). This effect is quite unusual, namely, in most cases the bathochromic shift occurs upon the interaction between the dye and fullerene due to the formation of a charge-transfer complex.[11,12] We believe that in our case the partial decrease in the polarization of the conjugated chain in the dye due to its interaction with the fullerene core can take place. The wavelength of fluorescence maximum of **2a** is equal to that of the IR-780 precursor, while for **2b** it is hypsochromically shifted by 55 nm. The value of Stokes shift for compound **2b** increases, which could be explained by the participation of the nitrogen atom in an excited-state intramolecular charge transfer.[8] For dyad **3a**, a hypsochromic shift of the fluorescence peak from 815 to 795 nm is detected compared to the precursor dye **2a**, while the wavelengths of the fluorescence maximum of dyad **3b** and dye **2b** coincide (see Figure 1 and Table 1). It is worth mentioning that both dyads have almost the same Stokes shift values,



Scheme 1 *Reagents and conditions*: i, 4-$HO_2CC_6H_4CHO$, sarcosine, *o*-$Cl_2C_6H_4$, 150 °C, overnight; ii, $HO(CH_2)_2SH$, $EtNPr^i_2$, CH_2Cl_2, room temperature, overnight (for **2a**); iii, $HO(CH_2)_2NH_2$, DMF, 70 °C, 4 h (for **2b**); iv, DCC, DMAP, THF, room temperature, 16 h.

compared to that of precursor dyes, namely, for dye **2a** and dyad **3a** they are 13 and 12 nm (Stokes shift is 199 and 192 cm^{-1}), and for dyad **3b** and dye **2b**, 108 and 99 nm (2180 and 1976 cm^{-1}), respectively. It could indicate the weak interaction of the dye with the fullerene core in both dyads.

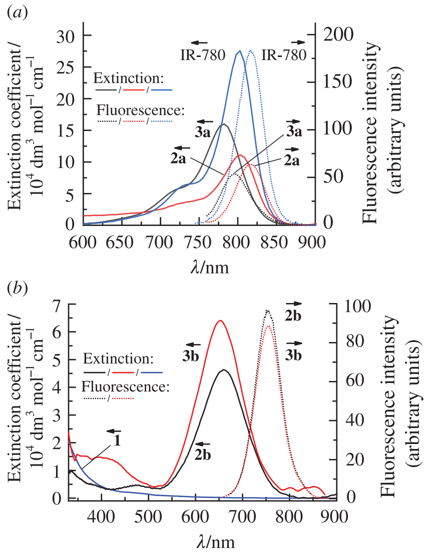


Figure 1 Absorption and fluorescence spectra of compounds (*a*) IR-780, **2a** and **3a** and (*b*) **1**, **2b** and **3b**. Fluorescence intensity was corrected on the absorbance at λ_{ex} for each compound (for **2a** λ_{ex} = 750 nm, for **3a** λ_{ex} = 750 nm, for **2b** λ_{ex} = 660 nm, and for **3b** λ_{ex} = 650 nm).

The fluorescence quantum yield of compounds under study was calculated by described method[10] using IR-780 as the reference compound (the Φ_F value of IR-780 as 8% EtOH solution was taken from reported data[9]). As it could be seen from Table 1, both dyes **2a**,**b** have a noticeably lower Φ_F compared to IR-780. In the case of **2b**, the lower Φ_F could be explained by the polarizing action of the nitrogen atom in the structure of the dye. The Φ_F values of dye **2b** and dyad **3b** practically coincide (17.5 and 16.1%), while for dyad **3a** it decreases by a factor of 3 compared to dye **2a** (see Table 1). The observed weak fluorescence quenching also indicates a weak interaction of the dye with the fullerene core for both dyads.

For compound **1**, a quasi-reversible reduction process was recorded at –1.19 V *versus* the ferrocene/ferricenium couple (see Online Supplementary Materials, Figure S8), which corresponded to the one-electron reduction of the fullerene core and it was close to the literature data for similar compounds.[13] In voltammograms of **2a**,**b**, quasi-reversible reduction processes were recorded at –1.07 (**2a**) and –1.32 V (**2b**), and oxidation at 0.11 (**2a**) and 0.17, 0.57 V (**2b**) (Figures S9 and S10). Also, for **2a**, **2b** irreversible peaks were recorded in the oxidative branch of voltammograms with a potential of –0.01 to –0.02 V which corresponded to the oxidation of the iodide anion. The obtained data allowed us to calculate the HOMO/LUMO energy levels (see Table 1). For dyad **3a**, a reversible reduction process was recorded at –1.08 V, and a weaker reversible process at –1.33 V; the oxidative process was recorded at 0.10 V(Figure S11). The process at –1.33 V probably corresponds to the second reduction potential of the fullerene moiety of the dyad. In contrast, the first

Table 1 Properties of the heptamethine cyanine–fullerene C_{60} dyads and their precursors.[a]

Compound	Absorbance λ_{max}/nm	Extinction coefficient ε at λ_{max}/dm^{-3} mol^{-1} cm^{-1}	Fluorescence λ_{max}/nm	Fluorescence quantum yield Φ_F (%)	Reduction/oxidation potential[b]/V	LUMO/HOMO level/eV
IR-780	803	284.8	815	36.8[c]	–/–	–/–
1	–	–	–	–	–1.19/–	–3.91/–
2a	802	111.2	815	12.9	–1.07/0.11	–4.03/–5.21
2b	660	46.4	759	17.5	–1.32/0.17	–3.78/–5.27
3a	783	159.9	795	4.1	–1.08/0.10	–4.02/–5.20
3b	652	64.1	760	16.1	–1.30/0.16	–3.80/–5.26

[a] The absorption and fluorescence properties of the compounds were measured in pyridine. Electrochemical and acid–base properties of **2a**,**b** and **3a**,**b** were measured in MeCN, for **1** in THF. [b] *vs*. Fc/Fc⁺. [c] Was taken for IR-780 (8% in ethanol) from ref. 9 and recalculated for pyridine using method.[10]

reduction peak of this fragment is masked by a more intense peak of the dye moiety. The significant difference in currents caused by the processes in the fullerene and cyanine parts of the molecule may be explained by the fact that in highly polar acetonitrile these dyads would form nanoparticles in which nonpolar fullerene spheroids are located inside the nanoparticles while the charged fragments of the dyes are placed outside, which makes the latter more accessible for redox processes. Such influence of solvent polarity on aggregation processes is well-known for various fullerene derivatives.[14] In the voltammogram of dyad **3b**, quasi-reversible reduction processes at –1.30 V and oxidation at 0.16 and 0.49 V were recorded (Figure S12). The values of the first oxidation and reduction potentials of dyads practically coincide with those for precursor dyes (see Table 1), which indicates the absence of influence of the fullerene core on the chromophores of dyes, which is in good agreement with photophysical data discussed above. It is interesting to note that for both dyads **3a**,**b**, in contrast to dyes **2a**,**b**, no peaks of oxidation of the iodide anion were observed. Apparently, it was replaced during the synthesis and purification by hydroxide and chloride anions, traces of which were recorded in mass spectra of dyads [Figure S2(*c*)].

Acid–base properties of compounds **2b** and **3b** have been explored. Compounds **2b** and **3b** bear an amine group in the spacer whose N–H acidic bond is strongly polarized (δ_{NH} value is 5.05 ppm). The ability of pH-dependent absorption change was shown previously[15] for fullerene–spiropyran dyads. In this regard, it is of interest to study the influence of the medium acidity on the properties of these dyes. The spectral changes of dye **2b** and dyad **3b** during both protonation and deprotonation have practically no differences (Figures S13 and S14). During deprotonation, a strong hypsochromic shift of the absorption band of the cyanine dye occurs (from 645 to 410 nm), indicating a significant disturbance of the conjugated bonds system of the dye. During protonation, a less strong hypsochromic shift of the absorption band occurs from 645 to 527 nm. The obtained stability constants for dye **2b** are given in Figures S13 and S14. For dyad **3b**, it was not possible to determine the stability constant of the protonated form due to the superposition of the dye and the pyrrolidine residue (which is located in the fullerene moiety) protonation processes.

A significant effect of fullerene C_{60} core on acid–base properties is known. For pyrrolidinofullerenes, for example, the basicity of the pyrrolidine nitrogen atom is significantly reduced due to the electron-acceptor effect of fullerene.[16] Furthermore, the absence of such an influence on the nitrogen atom of the dye (and its acid–base properties) in **3b** could be considered as another evidence of the weak interaction between the fullerene core and the dye moieties in the dyad.

A slight decrease in the acidity of dyad **3b** was found compared to dye **2b**, which may be associated with differences in the solvation of the precursor dye and dyad, as well as with aggregation processes. All acid–base changes with dye **2b** and dyad **3b** are entirely reversible. It should be noted that the neutral forms of dyad **3b** and dye **2b** exhibit intense fluorescence, while no fluorescence is observed in their protonated or deprotonated forms. This effect makes it possible to propose creating pH-sensitive fluorescent labels based on such structures.

When small amounts of DBU are added to **2a**, no spectral changes occur. However, at high concentrations of DBU, irreversible spectral changes arise, similar to those with **2b** (see Online Supplementary Materials, Figure S15), which indicates the destruction of the C–S bond with the formation of a compound structurally similar to the deprotonated form of dye **2b**. Similar behavior of **2a** is observed upon addition of trifluoroacetic acid: small amounts do not lead to a change in the absorption spectrum of the dye, and irreversible spectral changes occur at high acid concentrations (see Figure S15).

In conclusion, we have synthesized two new heptamethine cyanine dye–fullerene C_{60} dyads with pronounced absorption in the red and NIR spectral regions. An observed weak influence of the fullerene core on the photophysical and electrochemical properties of the dye in the structure of the dyads leads to weak quenching of the fluorescence. The fact that fullerenes act as extremely effective fluorescence quenchers[3,11] significantly complicates the use of the fluorescence-based methods of detection of fullerene derivatives and analysis of their biological activity. The dyads obtained seem promising for cell imaging and fluorescence diagnostics of various fullerene-based structures. The significant change of absorbance spectra and fluorescence properties upon protonation of NH-containing cyanine dyes can be applied in the design of pH-sensitive fullerene-containing fluorescent probes.

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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.2021.11.012.

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