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Design, synthesis and pH sensing properties of novel 1,8-naphtalimide-based bichromophoric system

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ABSTRACT

In this work we report on the design, synthesis and sensor properties of a novel bichromophoric sensor system based on 1,8-naphthalimide fluorophores. The synthesized dyad was configured as a fluorescent wavelength-shifting energy transfer chromophore. The novel donor–acceptor system contains blue emitting 4-methoxy-1,8-naphthalimide donor dye, capable of both absorbing light and efficiently transferring the energy to yellow-green emitting 4-*N*-methylpiperazinyl-1,8-naphthalimide acceptor. The energy-transfer efficiency in the dyad system was calculated to be more than 99%. The acceptor unit in the donor–acceptor system was also designed as a PET based sensor according to the "*fluorophore-spacer-receptor*" model. The fluorescence behaviour of the bichromophoric system was investigated as a function of pH. The fluorescence enhancement of the novel dyad in acidic media was more than 29 times indicating the high ability of the system to act as an efficient pH chemosensor.

1. Introduction

Fluorescent sensors are currently of great interest due to the increasing need of fast and reliable sensing of chemical species in many areas of human activity. With their intensive "naked eye detectable" fluorescent signal and high sensitivity, they allow immediate detection of protons [1–4], anions, cations and anions *in vivo* or in the environment [5,6], as well as detection of potentially dangerous substances like alkylation agents [7], organophosphorous compounds [8], chemical warfare [9], etc. Development of new sensors with improved characteristics (selectivity, sensitivity, etc.) is a challenging and necessary task in the 21st century, marked by the technological booms' consequences [10,11]. Different design strategies are being employed in the development of fluorescent sensors and variety of sensor systems which differ in their operation principle exist, for instance PET (photoinduced electron transfer) based sensors, CT (charge transfer) sensor, ET (electron transfer) sensors [12,13]. Combination of two design models in one single molecule could lead to profitable properties, such as improved selectivity and sensitivity, facilitated interpretation of sensor information, etc. [14].

A PET based fluorescent chemosensor involves a signal fragment (fluorophore) and a receptor, bound through a spacer ("*fluorophore-spacer-receptor*" model). When the receptor is unbound, the so assembled molecule looses its fluorescence due to photoinduced electron transfer (PET) from receptor to the fluorophore. Upon recognition of guest, which binds to the receptor, engaging its lone-pair electrons, the PET process is no longer possible and the fluorescence of the system is recovered [15].

1,8-Naphthalimide fluorophores are well studied class of luminophores possessing high photostability, excellent photophysical characteristics and have already found application as signal fragment in fluorescent sensor systems for protons [16–18], ions [19–21] and neutral molecules [22]. Previously, various PET naphthalimide based sensor systems, containing energy donor naphthalimides and single acceptor naphthalimide fragment have been reported [23–25]. The multichromophoric systems along with the excellent sensor properties, exhibit improved absorption characteristics (broader absorption band) and high energy transfer efficiency.

Considering the importance of PET systems and their growing application in a variety of areas, we were interested in the design, synthesis and photophysical investigation of a novel 1,8-naphthalimide PET based pH chemosensor. The new compound was designed as bichromophoric dyad containing 1,8-naphthalimide blue emitting donor dye which absorbs light and transfers its excitation energy to a yellow green-emitting 1,8-naphthalimide acceptor fluorophore. In addition, the acceptor dye was designed according to the "*fluorophore-spacer-receptor*" model, where the 4-amino-1,8-naphthalimide moiety is the fluorophore and the 4-methylpiperazine *N*-amine at C-4 is the receptor. Thus we expect that PET process from the tertiary piperazine amine

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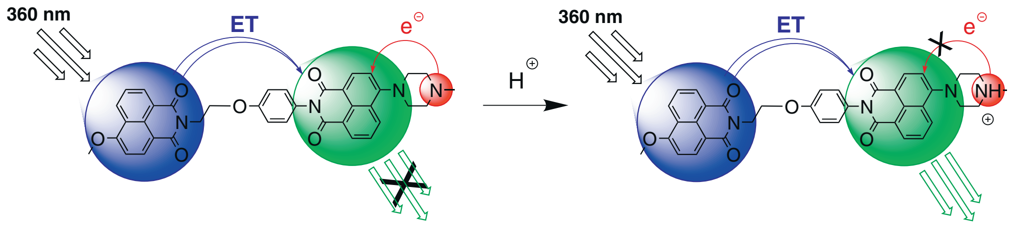


Scheme 1.

will effectively quench the yellow-green fluorescence of the acceptor representing the "*off*" state of the system. Upon recognition of guest, which binds to the receptor engaging its lone pair of electrons, PET communication between the receptor and the fluorophore would be cut off and the fluorescence of the system will recover (Scheme 1).

This article takes on added significance given the growing body of sensors and other optical devices which employ 1,8-naphthalimide fluorophores. Also we expect the distinguishing features of energy transfer wavelength-shifting chromophores that affords simultaneous recording of two emission intensities at different wavelengths in the presence and absence of analyte and provides a facile method for visualizing complex biological processes at the molecular level to be combined with the properties of classical PET systems.

2. Experimental

2.1. Materials

The starting 4-nitro-1,8-naphthalic anhydride **1** was prepared according to the reported procedure [26]. Ethanolamine, triphenylphosphine, tetrabromomethane, *p*-aminophenol and *N*-methylpiperazine (Fluka, Merck), p.a. grade, were used without purification. All solvents (Fluka, Merck) were pure or of spectroscopy grade.

2.2. Methods

FT-IR spectra were recorded on a Varian Scimitar 1000 spectrometer. The ^{1}H NMR spectra (chemical shifts are given as δ in ppm) were recorded on a Bruker DRX-250 spectrometer operating at 250.13 MHz. TLC was performed on silica gel, Fluka F60 254, 20 × 20, 0.2 mm. The melting points were determined by means of a Kofler melting point microscope. The UV–vis absorption spectra were recorded on a spectrophotometer Hewlett Packard 8452A. The fluorescence spectra were taken on a Scinco FS-2 spectrofluorimeter. The fluorescence quantum yields (Φ_F) were measured relatively to Coumarin 6 ($\Phi_F = 0.78$ in ethanol) [27] and p-methoxybenzylidenephthalide ($\Phi_F = 0.14$ in ethanol) [28].

2.3. Synthesis of blue emitting 1,8-naphthalimide donor dye 4

2.3.1. Synthesis of 4-nitro-N-(2-hydroxyethyl)-1,8-naphthalimide 2

Suspension of 4-nitro 1,8-naphthalic anhydride (5.3 g, 0.022 mol) and equimolar quantity of ethanolamine in ethanol was heated under reflux for 2 h. The hot reaction mixture was filtered to remove the unreacted starting anhydride and the filtrate was cooled to room temperature. The solid that precipitates was filtered off, washed with water and dried (5.01 g, 80%, m.p. 160–161 °C). FT-IR (KBr) cm^{-1}: 3416 (νOH); 2956 (ν^{as}CH$_2$); 2883 (ν^{s}CH$_2$); 1703 (ν^{as}N–$\underline{\text{C=O}}$); 1662 (ν^{s}N–$\underline{\text{C=O}}$); 1521 (ν^{as}NO$_2$); 1336 (ν^{s}NO$_2$). ^{1}H NMR (DMSO-*d*, 250.13 MHz) ppm: 8.66 (dd, 1H, *J* = 8.7 Hz, *J* = 0.9 Hz, naphthalimide H-7); 8.58 (dd, 1H, *J* = 7.5 Hz, *J* = 0.9 Hz, naphthalimide H-5); 8.54 (m, 2H, naphthalimide H-2, H-3); 8.05 (dd, 1H, *J* = 8.6 Hz, *J* = 7.5 Hz, naphthalimide H-6); 4.85 (m, 1H, OH); 4.14 (t, 2H, *J* = 6.4 Hz, $\underline{\text{CH}_2}$CH$_2$OH); 3.64 (m, 2H, CH$_2\underline{\text{CH}_2}$OH). Elemental analysis: calculated for $C_{14}H_{10}N_2O_5$ (MW 286.24) C 58.74, H 3.52, N 9.79%; found C 59.02, H 3.61, N 9.64%.

2.3.2. Synthesis of 4-methoxy-N-(2-hydroxyethyl)-1,8-naphthalimide 3

To a solution of 4-nitro-1,8-naphthalimide **2** (1 g, 0.0035 mol) in 4 ml of DMF a solution of 0.22 g KOH (0.004 mol) in 2 ml of methanol was added dropwise under stirring. The resulting mixture was stirred at room temperature for 2 h then poured into 10 ml of water. The precipitate was collected by filtration, washed with water and dried. The pure product was obtained after recrystallization from acetone (0.89 g, 75%, m.p. 184–186 °C). FT-IR (KBr) cm^{-1}: 3464 (νOH); 2957 (νCH$_3$); 2888 (νCH$_2$); 1693 (ν^{as}N–$\underline{\text{C=O}}$); 1649 (ν^{s}N–$\underline{\text{C=O}}$). ^{1}H NMR (CDCl$_3$-*d*, 250.13 MHz) ppm: 8.58 (m, 3H, naphthalimide H-7, H-5, H-2); 7.71 (dd, 1H, *J* = 8.4 Hz, *J* = 7.3 Hz, naphthalimide H-6); 7.07 (d, 1H, *J* = 8.3 Hz, naphthalimide H-3); 4.46 (m, 2H, $\underline{\text{CH}_2}$CH$_2$OH); 4.15 (s, 3H, O$\underline{\text{CH}_3}$); 4.00 (m, 2H, CH$_2\underline{\text{CH}_2}$OH); 2.46 (m, 1H, OH). Elemental analysis: calculated for $C_{15}H_{13}NO_4$ (MW 271.27) C 66.41, H 4.83, N 5.16%; found C 66.15, H 4.90, N 5.05%.

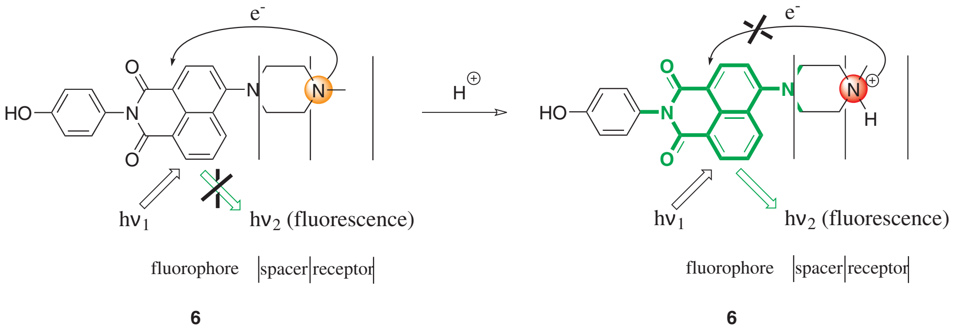
2.3.3. Synthesis of 4-methoxy-N-(2-bromoethyl)-1,8-naphthalimide 4

To a solution of 4-methoxy-1,8-naphthalimide **3** (0.35 g, 0.0013 mol) and tetrabromomethane (0.5 g, 0.0016 mol) in 5 ml of DMF 0.4 g (0.0016 mol) of triphenylphosphine were added. The mixture was stirred at room temperature for an hour then poured into 7 ml of water. The solid was filtered off, washed with water and dried (0.45 g, 74%, m.p. 190–192 °C). FT-IR (KBr) cm^{-1}: 2970 (νCH$_3$); 2933 (νCH$_2$); 1696 (ν^{as}N–$\underline{\text{C=O}}$); 1646 (ν^{s}N–$\underline{\text{C=O}}$). ^{1}H NMR (CDCl$_3$-*d*, 250.13 MHz) ppm: 8.60 (m, 2H, naphthalimide H-7, H-5); 8.56 (d, 1H, *J* = 8.3 Hz, naphthalimide H-2); 7.72 (dd, 1H, *J* = 8.4 Hz, *J* = 7.3 Hz, naphthalimide H-6); 7.06 (d, 1H, *J* = 8.3 Hz, naphthalimide H-3); 4.6 (t, 2H, *J* = 7.2 Hz, $\underline{\text{CH}_2}$CH$_2$OH); 4.15 (s, 3H, O$\underline{\text{CH}_3}$); 3.67 (t, 2H, *J* = 7.2 Hz, CH$_2\underline{\text{CH}_2}$Br). Elemental analysis: calculated for $C_{15}H_{12}BrNO_3$ (MW 334.16) C 53.91, H 3.62, N 4.19%; found C 54.19, H 3.54, N 4.31%.

2.4. Synthesis of yellow-green emitting 1,8-naphthalimide acceptor dye 6

A solution of 4-nitro-1,8-naphthalic anhydride (2.08 g, 0.0086 mol) and *p*-aminophenol (9.3 g, 0.0086 mol) in 50 ml of glacial acetic acid was stirred under reflux for 8 h. After cooling the precipitate was filtered off, washed with water and dried to give 1.93 g of pure 4-nitro-*N*-(4-hydroxyphenyl)-1,8-naphthalimide as brown crystals (1.93 g, 76%, m.p. >260 °C). FT-IR (KBr) cm^{-1}: 3413 (νOH); 1709 (ν^{as}N–$\underline{\text{C=O}}$); 1661 (ν^{s}N–$\underline{\text{C=O}}$); 1514 (ν^{as}NO$_2$); 1346 (ν^{s}NO$_2$). Elemental analysis: calculated for $C_{18}H_{10}N_2O_5$ (MW 334.28) C 64.67, H 3.02, N 8.38%; found C 64.92, H 2.95, N 8.50%.

To a solution of 0.96 g of 1,8-naphthalimide **5** (0.0029 mol) in 20 ml of DMF 0.29 g of *N*-methylpiperazine (0.0029 mol) were



Scheme 2.

added dropwise at room temperature. The resulting mixture was stirred for 168 h then poured into 30 ml of water. The crude product that precipitated was collected by filtration and dried. Recrystallization from chloroform afforded 0.56 g of yellow-green emitting naphthalimide **6** (0.56 g, 50%, m.p. >260° C). FT-IR (KBr) cm^{-1}: 3316 (νOH); 2807 (νCH$_2$); 1701 (ν^{as}N–$\underline{\text{C=O}}$); 1655 (ν^{s}N–$\underline{\text{C=O}}$), 1374 (νC–N). ^{1}H NMR (DMSO-*d*, 250.13 MHz) ppm: 9.63 (s, 1H, OH); 8.46 (m, 2H, naphthalimide H-7, H-5); 8.37 (d, 1H, *J* = 8.1 Hz, naphthalimide H-2); 7.78 (t, 1H, *J* = 7.9 Hz, naphthalimide H-6); 7.33 (d, 1H, *J* = 8.2 Hz, naphthalimide H-3); 7.09 (d, 2H, *J* = 8.8 Hz, Ph H-2, H-6); 6.82 (d, 2H, *J* = 8.7 Hz, Ph H-3, H-5); 3.24 (s, 4H, 2 × $\text{ArN}\underline{\text{CH}_2}\text{CH}_2\text{N}$); 2.65 (s, 4H, 2 × $\text{ArNCH}_2\underline{\text{CH}_2}\text{N}$); 2.31 (s, 3H, $\text{N}\underline{\text{CH}_3}$). Elemental analysis: calculated for $C_{23}H_{21}N_3O_3$ (MW 387.43) C 71.30, H 5.46, N 10.85%; found C 71.56, H 5.53, N 11.01%.

2.5. *Synthesis of donor–acceptor dyad* **7**

A mixture of 0.1 g of 1,8-napthalimide **6** (0.00025 mol), 0.086 g of blue-emitting 1,8-napthalimide **4** (0.00025 mol), dried potassium carbonate (0.018 g, 0.00013 mol) and TBAB (tetrabutylammonium bromide) was heated at 100 °C in 6 ml of dry DMF for 20 h under PTC (phase transfer catalysis) conditions. The solution was then poured into 15 ml of water and the precipitate was collected by filtration. Silica gel chromatography (chloroform:methanol = 9:1) afforded 0.025 g (15%) of dyad **7** as yellow crystals (m.p. 167–170 °C). ^{1}H NMR (CDCl$_3$-*d*, 250.13 MHz) ppm: 8.74 (dd, 1H, *J* = 8.4 Hz, *J* = 1.2 Hz, donor naphthalimide H-7); 8.69 (dd, 1H, *J* = 7.3 Hz, *J* = 1.2 Hz, donor naphthalimide H-5); 8.65 (dd, 1H, *J* = 7.3 Hz, *J* = 1.2 Hz, acceptor naphthalimide H-5); 8.58 (d, 1H, *J* = 8.1 Hz, donor naphthalimide H-2); 8.53 (d, 1H, *J* = 8.3 Hz, acceptor naphthalimide H-2); 8.49 (dd, 1H, *J* = 8.6 Hz, *J* = 1.2 Hz, acceptor naphthalimide H-7); 7.82 (dd, 1H, *J* = 8.4 Hz, *J* = 7.3 Hz, donor naphthalimide H-6); 8.75 (dd, 1H, *J* = 8.6 Hz, *J* = 7.3 Hz, acceptor naphthalimide H-6); 7.43 (d, 2H, *J* = 9 Hz, Ph H-2, H-6); 7.34 (d, 2H, *J* = 9 Hz, Ph H-3, H-5); 7.29 (d, 1H, *J* = 8.2 Hz, acceptor naphthalimide H-3); 7.16 (d, 1H, *J* = 8.2 Hz, donor naphthalimide H-3); 4.48 (m, 2H, $\underline{\text{CH}_2}\text{CH}_2\text{O}$); 3.67 (m, 2H, $\text{CH}_2\underline{\text{CH}_2}\text{O}$); 3.36 (m, 4H, $\text{ArN}\underline{\text{CH}_2}\text{CH}_2\text{N}$); 2.77 (m, 4H, $\text{ArNCH}_2\underline{\text{CH}_2}\text{N}$); 2.46 (s, 3H, $\text{O}\underline{\text{CH}_3}$); 1.25 (s, 3H, $\text{N}\underline{\text{CH}_3}$). Elemental analysis: calculated for $C_{38}H_{32}N_4O_6$ (MW 640.68) C 71.24, H 5.03, N 8.74%; found C 70.93, H 5.10, N 8.61%.

3. Results and discussion

3.1. *Design and synthesis of bichromophoric sensor dyad*

The new compound was designed as bichromophoric dyad containing 1,8-naphthalimide blue emitting donor dye which absorbs light and transfers its excitation energy to a yellow green-emitting 1,8-naphthalimide acceptor fluorophore. In order to obtain efficient energy transfer, maximal spectral overlap between the emission of the donor dye and the absorption of the acceptor dye is required. 1,8-Naphthalimide fluorophores poses high photostability and excellent photophysical characteristics which can be easily modified depending on the substituent nature at C-4 position. 4-Alkoxy substituted 1,8-naphthalimides have an absorption band around 360 nm and emission in the blue region where the absorption of the 4-amino substituted 1,8-naphthalimides appears [29–34]. Thus the combination of 4-alkoxy-1,8-naphthalimide as a donor and 4-amino-1,8-naphthalimides as an acceptor dye could provide satisfactory spectral overlap and an efficient energy transfer can be expected. Moreover, variety of such 1,8-naphthalimide-based systems with high energy transfer efficiency has already been reported [35,36]. Additionally, the acceptor unit in the novel dyad system was designed according to the "*fluorophore-spacer-receptor*" model, where the 4-amino-1,8-naphthalimide moiety is the fluorophore and the 4-methylpiperazine *N*-amine at C-4 position is the receptor. Thus we expect that photoinduced electron transfer (PET) from the tertiary amine in the piperazine substituent will effectively quench the fluorescence of the yellow-green emitting acceptor representing the "*off*" state of the system (Scheme 2). Upon recognition of guest, which binds to the receptor engaging its lone pair of electrons, PET communication between the receptor and the fluorophore would be cut off and the fluorescence of the system will be recovered [37].

For both donor and acceptor dyes the starting product was 4-nitro-1,8-naphthalic anhydride **1**. First, the blue emitting fluorophore **3** was obtained by reaction of 1,8-naphthalic anhydride with ethanolamine and subsequent substitution of the nitro group at C-4 position with methoxy one. The hydroxyethyl group was then converted to bromoethyl function upon treatment with Ph$_3$P and CBr$_4$ to afford fluorophore **4** (Scheme 3).

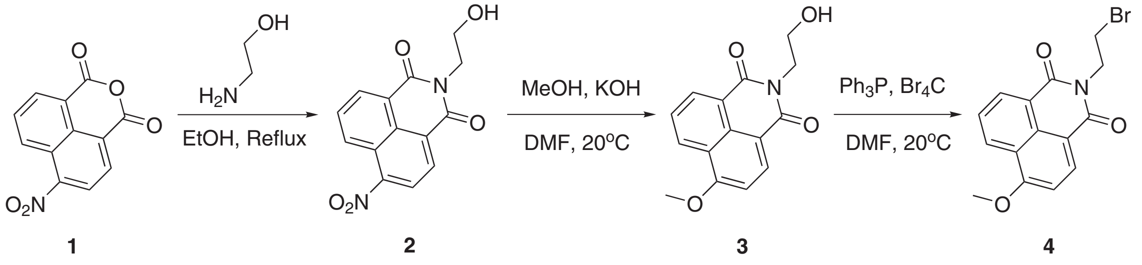
The acceptor dye **6** was prepared by reaction of **1** with *p*-aminophenol under reflux in acetic acid and subsequent introduction of a spacer-receptor fragment at 1,8-naphthalimide C-4 position (Scheme 4).

The dyad finally was assembled by Williamson reaction of the blue emitting 1,8-naphthalimide **4** and the acceptor dye **6** under PTC conditions (Scheme 5).

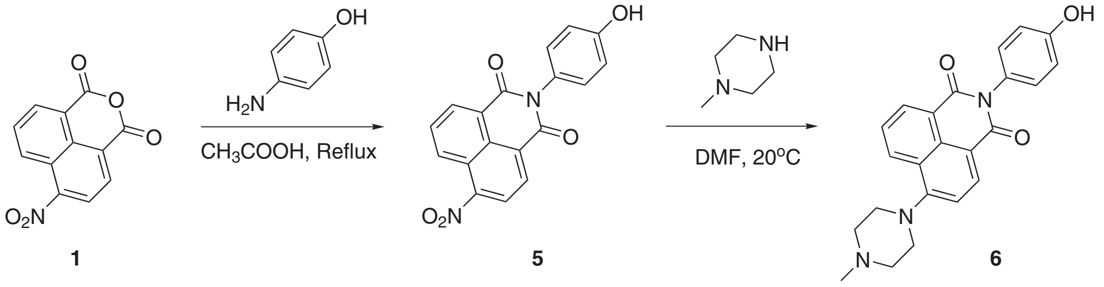
The synthesized compounds were characterized and identified by their melting points, TLC, elemental analysis data, UV–vis, fluorescence, FT-IR and ^{1}H NMR spectroscopy.

3.2. *Photophysical characterization of the dyes*

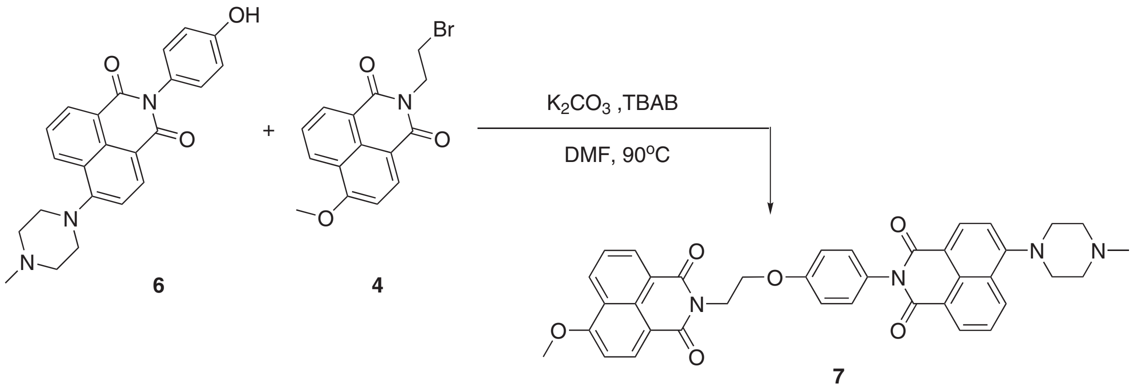
Photophysical properties of the 1,8-naphthalimide derivates are basically related to the polarization of their chromophoric system. Light absorption in this molecule generates a charge transfer interaction between the substituents at C-4 position and the imide carbonyl groups. In general, the derivatives with alkoxy groups are colorless and show blue emission, while the amino substituted



Scheme 3.



Scheme 4.



Scheme 5.

1,8-naphthalimides are yellow colored and display green fluorescence [29–34]. The absorption data of the examined compounds are listed in Table 1.

Compound **4** in chloroform, methanol and DMF is colorless with a typical for the 4-alkoxy substituted 1,8-naphthalimides maximal absorption at about 366 nm [38,39].

Acceptor fluorophore **6** exhibit in chloroform solution atypically blue shifted for the 4-aminosubstitued 1,8-naphthalimides absorption maximum at 388 nm. This phenomena has already been observed for some 4-aminoethylpipezinyl substituted 1,8-naphthalimides and it is possibly due to a repulsive interaction between the receptor tertiary amino group in the piperazine ring and the 4-amino moiety which destabilize the ICT excited state nature of this compound to a larger extent, resulting in more energy required to access the excited state [40,41]. Compound **6** also shows typical for ICT fluorophores bathochromic shift of the longest-wavelength band upon increasing the solvent polarity (λ_A = 396 nm in methanol and λ_A = 398 nm in DMF).

Table 1
Absorption data of dyad **7** and 1,8-naphthalimides **4** and **6** in different solvents.

Compound	Chloroform	Methanol	DMF	
	λ_A (nm)	λ_A (nm)	λ_A (nm)	ε (l mol^{-1} cm^{-1})
Donor dye **4**	366	366	364	16,902
Acceptor dye **6**	388	396	398	15,981
Dyad **7**	366	372	374	21,457

Dyad **7** shows a more complex absorption spectrum which as expected contains absorption bands corresponding to the absorptions of donor and acceptor dyes **4** and **6** (Fig. 1). Therefore it can be assumed that there is no interaction between the chromophores in the system and the absorption properties of the fluorophores in the dyad do not differ from single donor and acceptor fluorophores' properties.

All of the compounds under study showed in the longest-wavelength absorption band typical for the 1,8-naphthalimide dyes molar extinction coefficients (ε), higher than 10,000 l mol^{-1} cm^{-1}, indicating that this is a charge transfer (CT) band [42,43].

The fluorescent characteristics of the 1,8-naphthalimides **4**, **6** and dyad **7** in different solvents such as fluorescence maxima (λ_F), Stokes shift (ν_A–ν_F) and fluorescence quantum yield (Φ_F), are presented in Tables 2 and 3.

In solution compound **4** display blue fluorescence due to the charge transfer from the electron-donating group at 1,8-naphthalimide C-4 position to the carbonyl groups. Compound **6**

Table 2
Fluorescence characteristics of 1,8-naphthalimides **4** (λ_{ex} = 360 nm) and **6** (λ_{ex} = 420 nm) in different solvents.

Compound	Chloroform			Methanol			DMF		
	λ_F (nm)	$\nu_A - \nu_F$ (cm^{-1})	Φ_F	λ_F (nm)	$\nu_A - \nu_F$ (cm^{-1})	Φ_F	λ_F (nm)	$\nu_A - \nu_F$ (cm^{-1})	Φ_F
Donor dye **4**	430	4067	0.521[a]	449	5050	0.453[a]	440	4745	0.423[a]
Acceptor dye **6**	505	5971	0.185[b]	543	6836	0.012[b]	529	6222	0.005[b]

[a] Quantum yields calculated using and *p*-methoxybenzylidenephthalide (Φ_F = 0.14 in ethanol).
[b] Quantum yields calculated using Coumarin 6 (Φ_F = 0.78 in ethanol).

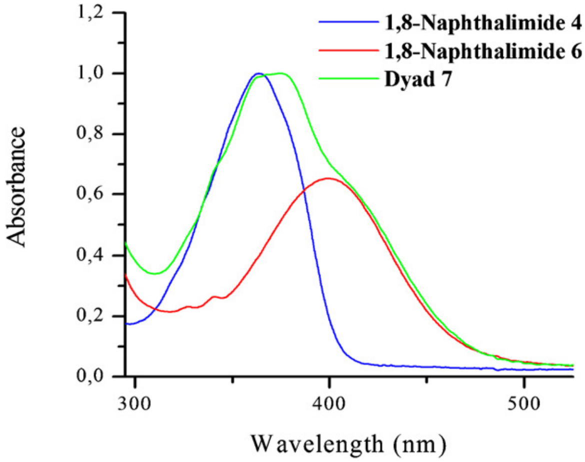


Fig. 1. Normalized absorption spectra of 1,8-naphthalimides **4**, **6** and dyad **7** in DMF.

showed typical for the 4-piperazinyl-1,8-naphthalimides yellow-green fluorescence [44] due to the stronger electron-donating ability of the C-4 substituent.

The Stoke's shift ($\nu_A - \nu_F$) is important parameter for the fluorescent compounds that indicates the differences in the properties and structure of the fluorophores between the ground state S_0 and the first excited state S_1. The Stoke's shift values (cm^{-1}) were calculated by Eq. (1).

$$(\nu_A - \nu_F) = \left(\frac{1}{\lambda_A} - \frac{1}{\lambda_F}\right) \times 10^7 \qquad (1)$$

The Stoke's shift values of compound **4** are between 4067 cm^{-1} and 5050 cm^{-1} in different solvents and correspond to the results for other 4-alkoxy-1,8-naphthalimide derivatives [29–31].

The Stoke's shift values for acceptor 1,8-naphthalimide **6** (5971–6836 cm^{-1}) are relatively higher but also typical for the 4-piperazine substituted 1,8-naphthalimides [40,41]. As the dipole moment of the molecule is enhanced upon excitation due to electron density redistribution, the excited molecule is better stabilized in polar solvents, such as DMF and methanol, because of stronger interaction with the solvent dipoles [40,45]. This effect causes the red shift in the fluorescence maxima resulting in large-scale augmentation of the Stoke's shift. The Stokes shift values of the dyes under study do not indicate remarkable changes in the geometry of the first singlet excited state in comparison with other naphthalimide fluorophores. Due to considerable absorption overlap between the donor and acceptor units in dyad **7** it is difficult to discuss the Stoke's shift values of the latter. However the absorption spectrum of **7** consists of two bands, corresponding to the absorptions of single fluorophores **4** and **6** indicating no interaction between the chromophores in the molecule.

Table 3
Fluorescence characteristics of dyad **7** in different solvents.

Chloroform	Methanol	DMF	
λ_F (nm)	λ_F (nm)	λ_F (nm)	Φ_F
504	525	527	0.01

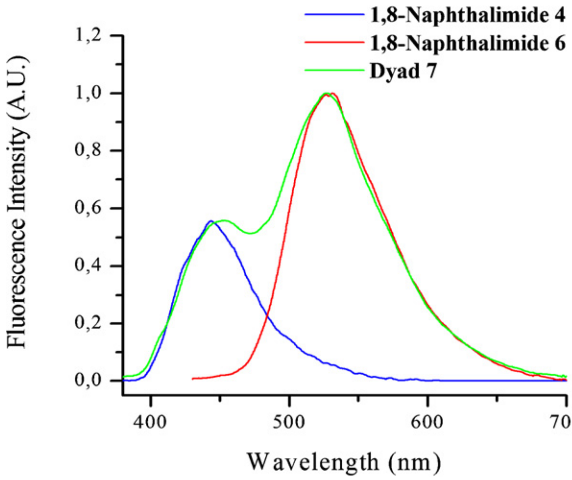


Fig. 2. Normalized fluorescent spectra of naphthalimide **4** (λ_{ex} = 360 nm), **6** (λ_{ex} = 420 nm) and dyad **7** (λ_{ex} = 360 nm) in DMF.

The fluorescence spectra of the dyad **7** in DMF solution, obtained after excitation within the spectral region of maximal absorption of the donor fluorophore (λ_{ex} = 360 nm), showed two emission bands, corresponding to the emission bands of donor and acceptor 1,8-naphthalimide fragments in the donor–acceptor systems (Fig. 2).

As a result of the energy transfer, the emission intensity of the donor (blue emitting donor 1,8-naphthalimide fragment) in the systems was strongly decreased in comparison to the single blue emitting fluorophore **4**. This is illustrated in Fig. 3, where are presented the recorded at the same optical density fluorescence spectra of dyad **7** and donor dye **4**, excited within the maximal absorption of the donor (λ_{ex} = 360 nm).

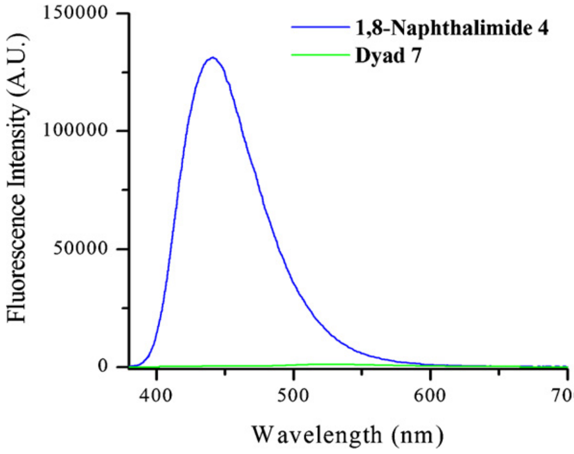


Fig. 3. Normalized to the optical density fluorescence spectra of naphthalimide **4** and dyad **7** in DMF excited at 360 nm.

The energy-transfer efficiency (E_T) in the donor–acceptor system was calculated to be more than 99% using Eq. (2) [46], where F_D is the normalized to the optical density fluorescence intensity of donor model compound **4** and F_{DA} is the normalized to the optical density fluorescence intensity of the donor chromophore in dyad **7**. Since the energy transfer efficiency is strongly dependent on distance between donor and acceptor fragments, such a high energy transfer in dyad **7** was predictable.

$$E_T = 1 - \left(\frac{F_{DA}}{F_D}\right) \tag{2}$$

The ability of molecules to emit the absorbed light is characterized quantitatively by the fluorescence quantum yield (Φ_F). The quantum yields of fluorescence were calculated using Coumarin 6 ($\Phi_F = 0.78$ in ethanol) for the yellow-green emitting 1,8-napthalimide **6** and dyad **7** and *p*-methoxybenzylidenephthalide ($\Phi_F = 0.14$ in ethanol) for the blue emitting dye **4**, as a standards according to Eq. (3) [47]. A_{ref}, S_{ref}, n_{ref} and A_{sample} S_{sample}, n_{sample} represent the absorbance at the excitation wavelength, the integrated emission band area and the solvent refractive index of the standard and the sample, respectively.

$$\Phi_F = \Phi_{ref}\left(\frac{S_{sample}}{S_{ref}}\right)\left(\frac{A_{ref}}{A_{sample}}\right)\left(\frac{n^2_{sample}}{n^2_{ref}}\right) \tag{3}$$

The quantum yield data are presented in Tables 2 and 3. As can be seen the sensor compounds **6** and **7** have low quantum yield compared to the traditional 4-amino-1,8-naphthalimides, not containing amine receptor [47]. This phenomenon might be caused by the possible photoinduced electron transfer from the *N*-methylpiperazine amine receptor to the 4-amino-1,8-naphthalimide fluorophore through the saturated piperazinyl ring (Scheme 2). Thus the fluorescence of the 4-amino-1,8-naphthalimide fluorophore is quenched. On the other hand the quantum yield of methylpiperazinyl substituted naphthalimide **6** is highly solvent dependent. In polar media the quantum yield is very low ($\Phi_F = 0.005–0.012$), while in non-polar media the quantum yield is considerably higher ($\Phi_F = 0.185$). This fact indicates PET process which is quenched in non-polar media causing restoration of the fluorescence emission [36]. Upon recognition of the analyte (protons) *N*-methylpiperazine amine would increase the oxidation potential of the receptor, and as such, thermodynamically disallow the electron transfer and the emission would be “switched on”.

3.3. Influence of pH on absorption and fluorescent properties of the dyes

The novel compound **7** was designed as bichromophoric sensor system for detection of pH changes over a wide interval. Therefore it was of interest to study the photophysical behaviour of the new compound **7** in water/DMF (4:1 v/v) solution as a function of pH. In order to receive a more complete comparative picture for the properties of the bichromophoric dyad compounds **4** and **6**, representing respectively the single donor and acceptor fragment, were also included in this investigation as reference compounds.

The absorption spectra of blue emitting naphthalimide **4** in water/DMF (4:1 v/v) at different pHs are presented in Fig. 4. As expected the compound showed no changes in the absorption maximum and the absorption profile indicating no proton influence on the absorption properties. In contrast to **4**, sensor **6** showed a blue shifted absorption maxima of about 20 nm upon acidification (Fig. 5). One of the reasons for this effect is that the protonation of the amine receptor exerts some weak charge repulsion on the 4-amino moiety of the fluorophores. On the other hand in very acidic conditions the push–pull character of the ICT state is partially reduced due to the protonation of the aromatic 4-amino moiety [40].

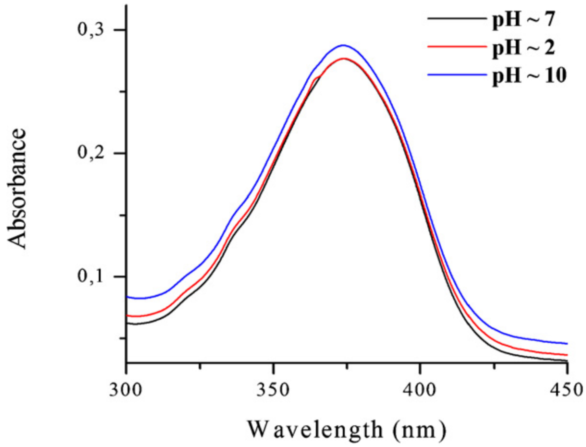


Fig. 4. Absorption spectra of compound **4** in water/DMF (4:1 v/v) at different pH values.

Similar to the absorption properties, the fluorescent characteristics of blue-emitting fluorophore **4** in water/DMF (4:1 v/v) remain unchanged in a wide pH interval. In contrast to **4** compounds **6** and **7** showed significant changes in the emission intensity at different pH values. Family of fluorescent spectra ($\lambda_{ex} = 420$ nm) of compound **6** at different pH values in water/DMF (4:1 v/v) are presented in Fig. 6. As expected in alkaline media the compound showed very weak fluorescence which was increasing gradually upon acidification. The increment of the fluorescent intensity together with the lower quantum yield in polar media than in nonpolar one (Table 2) indicates that the fluorescence of compound **6** is being quenched by PET process from the tertiary amino group to the fluorophore. Upon recognition of the analyte (protons) the oxidation potential of the receptor is being increased thermodynamically disallowing PET process and the fluorescence of the compound is recovered.

The changes of the fluorescence intensity ($\lambda_{ex} = 420$ nm) of compound **6** as a function of pH in water/DMF (4:1 v/v) are plotted in Fig. 7. After careful titration from pH *ca.* 10 to pH *ca.* 2 the fluorescence of the yellow-green emitting naphthalimide **6** had enhanced more than 50 times (FE = 52). The results show that the fluorophore switches between “*off*” and “*on*” state in the interval of pH *ca.* 8.5–6.5.

Taking part of the graph between pH 4 and pH 10 the pK_a value of sensor **6** was calculated using Eq. (4) [18]. The pK_a value was calcu-

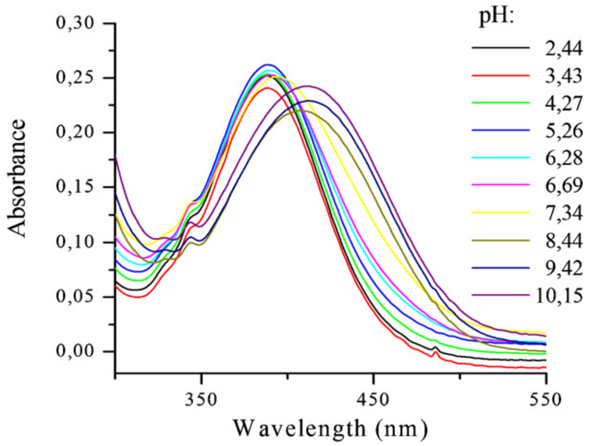


Fig. 5. Absorption spectra of compound **6** in water/DMF (4:1 v/v) at different pH values.

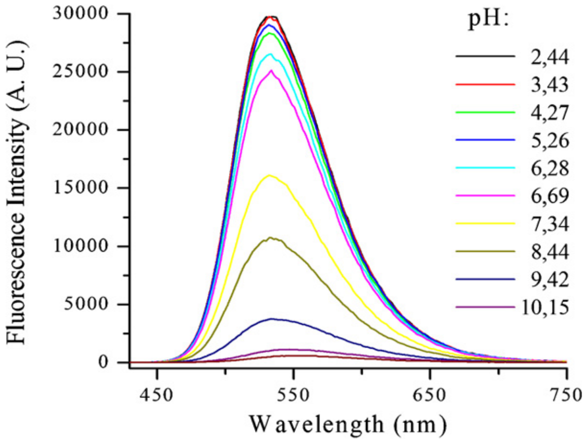


Fig. 6. Fluorescence spectra of **6** in water/DMF (4:1 v/v) at different pH values (λ_{ex} = 420 nm).

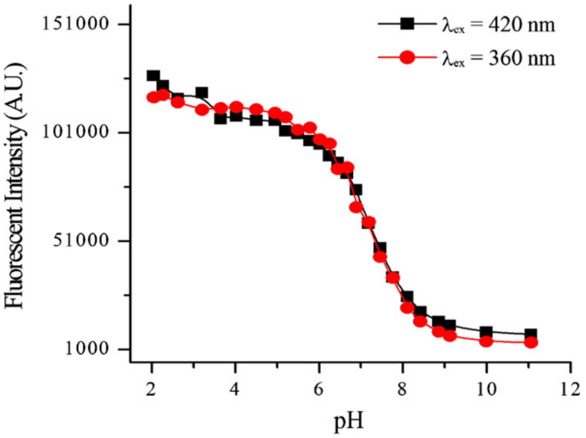


Fig. 9. Normalized to the same optical density fluorescence intensity of dyad **7** (λ_{em} = 535 nm) in water/DMF (4:1 v/v) as a function of pH.

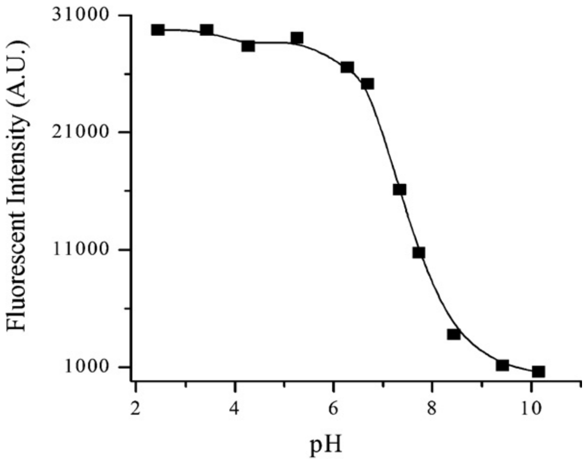


Fig. 7. Effect of pH on the fluorescence intensity (λ_{ex} = 420 nm, λ_{em} = 535 nm) of compound **6** in water/DMF (4:1 v/v).

lated to be 7.45 which is in agreement with the data of previously reported compounds of the same nature [44]. The pK_a calculated by means of the variation of the absorption spectra as a function of pH comes up to 7.87, suggesting absorption changes caused by the ionization of the phenoxy group in alkaline media, which increases the solubility of the compound in the solvent system water/DMF.

$$\log\left[\frac{I_{\mathrm{F\,max}}-I_{\mathrm{F}}}{I_{\mathrm{F}}-I_{\mathrm{F\,min}}}\right] = \mathrm{pH}-\mathrm{p}K_{\mathrm{a}} \qquad (4)$$

The changes of the fluorescence intensity of the acceptor fluorophore in dyad **7** as a function of pH was recorded in water/DMF (4:1 v/v) and are presented in Fig. 8A and B. In alkaline media the fluorescence of the acceptor unit is quenched due to PET from the tertiary amine in the receptor to the fluorophore. Upon recognition of guest, which binds to the receptor engaging its lone pair of electrons, PET communication between the receptor and the fluorophore is cut off and the fluorescence of the system is recovered (Scheme 2). The acceptor fluorescence intensity of dyad **7** in acidic media excited by the energy transfer from the donor unit (λ_{ex} = 360 nm) is app. 1.5 times higher than the fluorescence intensity of the compound excited directly in the acceptor (λ_{ex} = 420 nm). This fact is a result of the higher light input in the system due to higher absorption ability of dyad **7** at 360 nm and is very well correlated with the difference in the optical density of compound **7** at 360 and 420 nm.

The changes in the fluorescence intensity of the receptor unit in dyad **7** in water/DMF (4:1 v/v) as a function of pH after excitation at 360 and 420 nm at the same optical density are presented in Fig. 9. The titration curves presented in figure are very similar, showing that pH sensing properties of the dyad are related only to the sensitivity of the acceptor fluorophore, which contains PET quenching

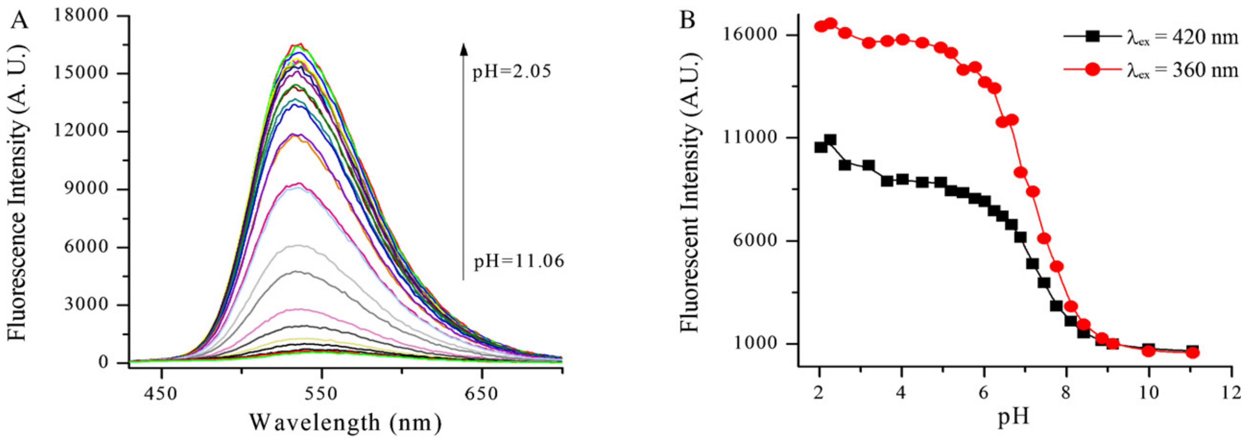


Fig. 8. (A) Fluorescence spectra of **7** in water/DMF (4:1 v/v) at different pH values (λ_{ex} = 360 nm). (B) Effect of pH on the fluorescence intensity of compound **7** (λ_{em} = 535 nm) in water/DMF (4:1 v/v).

fragment. Upon acidification the tertiary amine (receptor) is being protonated and the yellow-green fluorescence of the acceptor fluorophore is increasing gradually. Moreover the titration profile of the curve in Fig. 8B, obtained after direct excitation of the acceptor fragment (λ_{ex} = 420 nm), is a very similar to that of the curve, registered after normalization to the same optical density (Fig. 9), which suppose lack of absorption changes during the titration. The acceptor's fluorescence enhancement of dyad **7** in acidic media was calculated to be more than 29 times, demonstrating the ability of the novel compound to act as a highly efficient pH sensor.

Taking part of the curves plotted in Fig. 9 between pH 4 and pH 10 the pK_a value for compound **7** was calculated by Eq. (4). The calculated pK_a value (pK_a = 7.12) differs from the emission pK_a value of compound **6** (pK_a = 7.45). The alkaline shifted value of **6** could be related to the presence of aromatic hydroxyl group in the molecule resulting in better solubility of the compound in alkaline media.

4. Conclusion

A novel fluorescent donor–acceptor system with PET sensing properties, containing blue emitting 4-methoxy-1,8-naphthalimide donor and yellow-green emitting 4-*N*-methylpiperazinyl-1,8-naphthalimide acceptor, was synthesized. The novel compound was designed as a wavelength-shifting energy transfer chromophore capable of absorbing light by its donor chromophore and efficiently transferring the energy to the acceptor chromophore of the system. The synthesized dyad system showed highly efficient energy transfer (more than 99%) suggesting that the selected 1,8-naphthalimides are suitable donor–acceptor pair for energy transfer systems. The novel compound was also designed according to the "*fluorophore-spacer-receptor*" model and as such it would be able to act as a fluorescence PET based chemosensor. The fluorescent enhancement of the dyad system in acidic media is more than 29 times, demonstrating the high potential of the compound to act as an effective "*off–on*" switch for pH. Thus the distinguishing features of energy transfer wavelength-shifting chromophores were successfully combined with the properties of classical PET systems.

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