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A highly selective ratiometric fluorescent pH probe based on a PAMAM wavelength-shifting bichromophoric system

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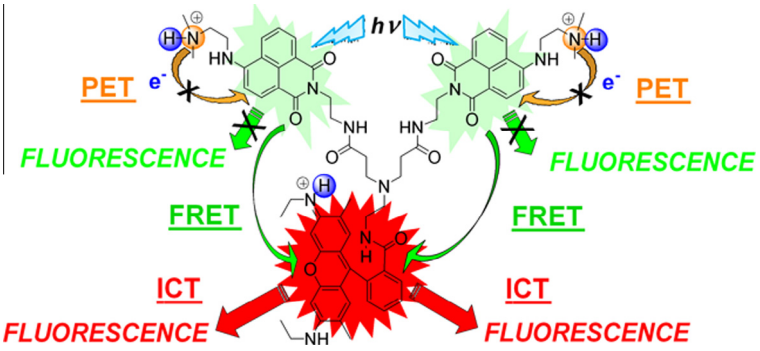
HIGHLIGHTS

- PAMAM antenna decorated with Rhodamine 6G and 1,8-naphthalimides is synthesized.
- Periphery of the antenna is designed as a PET based fluorescence probe.
- System manifests excellent selective response to protons in aqueous medium.
- Core emission of the systems is enhanced 30 times as a function of pH.
- Bichromophoric system acts as a selective ratiometric probe in complex samples.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel PAMAM wavelength-shifting bichromophoric system has been successfully developed. Novel compound was configured as a light harvesting antenna where the system surface is labeled with yellow–green emitting 4-(*N*,*N*-dimethylamino)ethylamino-1,8-naphthalimide "donor" units capable of absorbing light and efficiently transferring the energy to a focal Rhodamine 6G "acceptor". The periphery of the system was designed on the "fluorophore–spacer–receptor" format, capable of acting as a molecular fluorescence photoinduced electron transfer based probe. Due to the both effects, photoinduced electron transfer in the periphery of the system and pH dependent rhodamine core absorption, novel antenna is able to act as a selective ratiometric pH fluorescence probe in aqueous medium. Thus, the distinguishing features of the fluorescence resonance energy transfer systems were successfully combined with the properties of classical ring-opening charge transfer systems, which may be beneficially for monitoring pH variations in complex samples.

Introduction

The detection and control of chemical constituents comprised of different analytes has become an indispensable task in many applications related to the management of minimum standards of foods, environment, agriculture sciences, medicine and health sciences [1–4]. Because of the high sensitivity, high speed and cheap instrumentation, particularly the fluorescence sensors and switches have been actively investigated [5,6]. They are designed on three basic approaches – intramolecular charge transfer (ICT), photoinduced electron transfer (PET) and energy transfer [7–9].

The most reported fluorescent sensors display an increase or decrease in the emission intensity upon binding to species of

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interest. As the change in fluorescence intensity is the only detection signal, factors such as instrumental efficiency, environmental conditions, and the probe concentration can interfere with the signal output. To eliminate these effects, a ratiometric fluorescent measurement is desirable. The ratiometric fluorescent probes emit fluorescence at two wavelengths that enable a built-in correction for the undesired environmental effects. They operate via dual output signaling of the ICT [10,11], excited-state intramolecular proton transfer (ESIPT) [12] or bichromophoric systems [13].

Among the ratiometric fluorescent probes, we were interested in developing new bichromophores with fluorescence sensing properties, based on fluorescence resonance energy transfer (FRET). FRET is a distance-dependent interaction between the electronic excited states of two dye molecules in which excitation is transferred from a donor molecule to an acceptor molecule without emission of a photon [14]. The pseudo-Stokes shifts of FRET based probes are larger than the Stokes shifts of either the donor or acceptor dyes, thus, the possible self-quenching as well as fluorescence detection errors due to backscattering effects from the excitation source will be efficiently avoided [15].

The most attractive artificial light-harvesting systems are the dendritic assemblies because of their unique structures, reminiscent of the architecture of natural light-harvesting complexes [16,17]. The globular shape of dendritic architectures provides a large surface area that can be decorated with chromophores, resulting a large absorption cross section and efficient capture of photons. Furthermore, because of their proximity, the various functional groups of dendritic systems may easily interact with one another to give high efficiency energy transfer [18]. The polyamidoamines (PAMAM) are a well know class of commercial dendrimers. The use of flexible aliphatic PAMAM bone as a scaffold for light-harvesting antennae could give new systems with high efficiency of energy transfer [18,19].

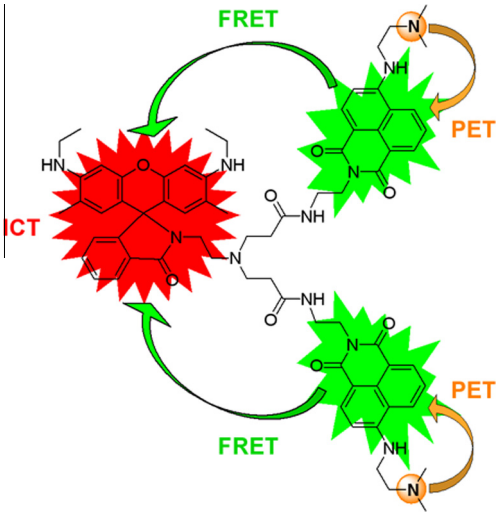
Particularly useful for wavelength-shifting bichromophoric systems are fluorophores such as Rhodamine 6G and 1,8-naphthalimide [20–26]. Because of their excellent fluorescence properties and good photostability, 1,8-naphthalimide dyes were used extensively in a number of areas, including chemosensing materials [27–43]. On the basis of the spirolactam (non-fluorescent) to ring-open amide (fluorescent) equilibrium of rhodamine, series of rhodamine-based dyes with excellent "off–on" switching of fluorescence upon encountering the correct target have been synthesized [44–47].

In this paper, we report on the design, synthesis and photophysical properties of a novel ratiometric fluorescence "off–on" light-harvesting antenna based on a core and peripherally functionalized PAMAM dendron (Scheme 1). The decorated with yellow–green emitting 1,8-naphthalimide units dendron periphery is capable of absorbing light and efficiently transferring the energy to a focal Rhodamine 6G. Also, the peripheral 1,8-naphthalimides were designed on a "fluorophore–spacer–receptor" format thus providing PET based sensing properties of the novel light harvesting antenna.

Experimental

Materials

Commercially available Rhodamine 6G **1**, methyl acrylate, ethylenediamine and *N*,*N*-dimethylethylenediamine (Aldrich, Merck) were used without purification. The starting 4-nitro-1,8-naphthalic anhydride **5** and the intermediate dendron **4** were prepared according to the reported procedures [21,48]. All solvents (Fluka, Merck) were pure for analysis or of spectroscopy grade. NaOH and HCl were supplied by Merck (Germany). HEPES buffer solution



Scheme 1. PAMAM light-harvesting antenna **7**, core and peripherally functionalized with Rhodamine 6G and 4-(*N*,*N*-dimethylamino)ethylamino-1,8-naphthalimides.

(pH 7.3, Aldrich), metal stock solutions (1×10^{-3} M) of $Zn(NO_3)_2$, $Cu(NO_3)_2$, $Ni(NO_3)_2$, $Co(NO_3)_2$, $Pb(NO_3)_2$, $Fe(NO_3)_3$, $Cd(NO_3)_2$, $AgNO_3$ and $Hg(NO_3)_2$ in DMF (all Aldrich salts at p.a. grade) and working dye solutions (1×10^{-5} M) were prepared daily.

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. Absorption spectra were recorded on a Hewlett Packard 8452A spectrophotometer. Fluorescent spectra were recorded on a Scinco FS-2 fluorescence spectrophotometer. The excitation source was a 150 W Xenon lamp. Excitation and emission slits width were 5 nm. Fluorescence measurement was carried out in right angle sample geometry. A 1 × 1 cm quartz cuvette was used for the spectroscopic analysis. Relative fluorescence quantum yields (Φ_F) were measured using Rhodamine 6G (Φ_F = 0.95 in ethanol [49]) or Coumarin 6 (Φ_F = 0.78 in ethanol [50]) as standards. All experiments were performed at room temperature. The spectral data were collected using FluoroMaster Plus 1.3 and further processed by OrginPro 6.1 software. A pH meter Metrohm 704 coupled with combined pH electrode was used for pH measurements. The commercial standard buffers for pH 2, 7 and 10 (Aldrich) were used for calibration. TLC was performed on silica gel, Fluka F60 254, 20 × 20 cm, 0.2 mm. The melting points were determined by means of a Kofler melting point microscope.

The absorption and fluorescence properties were studied as a function of pH by multiple additions of NaOH and HCl aqueous solutions to 400 mL 1×10^{-5} M solution of examined compounds in water/DMF (4:1, v/v). The addition was limited to 1 mL so that dilution remains insignificant. The solution pH, absorption and fluorescence spectra were recorded at each addition. The effect of the metal cations was examined by adding 10 μL of the metal stock solutions to 4 mL of the buffered water/DMF (4:1, v/v) fluorophore solution (1×10^{-5} M).

Synthesis of rhodamine core (*2*)

Ethylenediamine (3.6 mL, 56 mM) was added dropwise to a solution of Rhodamine 6G **1** (4.4 g, 9.2 mM) in 120 mL of absolute ethyl alcohol at room temperature. The resulting solution was

stirred at reflux for 5 h. After cooling to room temperature the solid precipitated was filtered off, washed with water and dried to give 3.57 g (85%) of **2** as pale pink crystals. FT-IR (KBr) cm^{-1}: 3218 (νNH and $νNH_2$); 2936, 2842 (νCH); 1682 (νC=O); 1618, 1522 and 1480 (νAr=CH). ^1H NMR ($CDCl_3$-*d*, 250.13 MHz) ppm: 7.88 (m, 1H, 9-Ph H-3); 7.44 (m, 2H, 9-Ph H-4 and 9-Ph H-5); 7.01 (m, 1H, 9-Ph H-6); 6.35 (s, 2H, Rhodamine H-4 and H-5); 6.19 (s, 2H, Rhodamine H-1 and H-8); 3.62 (br.s, 2H, 2 × ArNH); 3.20 (m, 6H, 2 × CH_2CH_3 and CH_2NCO); 2.34 (t, 2H, *J* = 6.9 Hz, CH_2NH_2); 1.91 (s, 6H, 2 × $ArCH_3$); 1.31 (m, 8H, 2 × CH_2CH_3 and NH_2). Elemental analysis: Calculated for $C_{28}H_{32}N_4O_2$ (MW 456.58) C 73.66, H 7.06, N 12.27%; Found C 73.38, H 6.98, N 12.08%.

*Synthesis of ester-functionalized dendron (**3**)*

A solution of methyl acrylate (6.75 mL, 75 mM) in 8 mL of methanol was added dropwise over a period of 30 min to a suspension of **2** (3.42 g, 7.5 mM) in 60 mL of cooled to 0°C methanol. The reaction mixture was allowed to warm slowly to room temperature and then stirred for 3 days. The ester-terminated dendron **3** was isolated as white crystals after evaporation of methyl acrylate and methanol under vacuum in yield 4.53 g (96%). FT-IR (KBr) cm^{-1}: 3322 (νNH); 2938, 2890 (νCH); 1732 (νCOOMe); 1674 (νC=O); 1622, 1520 and 1462 (νArCH). ^1H NMR ($CDCl_3$-*d*, 250.13 MHz) ppm: 7.90 (m, 1H, 9-Ph H-3); 7.46 (m, 2H, 9-Ph H-4 and 9-Ph H-5); 7.03 (m, 1H, 9-Ph H-6); 6.36 (s, 2H, Rhodamine H-4 and H-5); 6.20 (s, 2H, Rhodamine H-1 and H-8); 3.58 (s, 6H, 2 × OCH_3); 3.37 (br.s, 2H, 2 × ArNH); 3.18 (q, 4H, *J* = 7.1 Hz, 2 × $ArNHCH_2$); 2.58 (t, 4H, *J* = 7.2 Hz, 2 × CH_2COOCH_3); 2.22 (t, 2H, *J* = 7.2 Hz, CH_2NCOAr); 2.16 (m, 6H, $N(CH_2)_3$); 1.88 (s, 6H, 2 × $ArCH_3$); 1.33 (t, 6H, *J* = 7.1 Hz, 2 × CH_2CH_3). Elemental analysis: Calculated for $C_{36}H_{44}N_4O_6$ (MW 628.76) C 68.77, H 7.05, N 8.91%; Found C 68.53, H 7.11, N 9.02%.

*Synthesis of amino-terminated dendron (**4**)*

To a suspension of ester-functionalized rhodamine **3** (4.40 g, 7 mM) in 25 mL of methanol a solution of ethylenediamine (29 mL, 420 mM) in 20 mL of cooled to 0°C methanol, was added over a period of 30 min. The resulting mixture was stirred for 7 days at room temperature. Then the solvent and the ethylenediamine excess were distilled under vacuum. Final traces of excess ethylenediamine were removed azeotropically using a 9:1 toluene/methanol (v/v) solution. The amino-terminated dendron **4** was obtained as white crystals in yield 4.51 g (94%). FT-IR (KBr) cm^{-1}: 3316 and 3208 (νNH and $νNH_2$); 2924, 2882 (νCH); 1646 (νC=O); 1632 and 1484 (νAr=CH). ^1H NMR ($CDCl_3$-*d*, 250.13 MHz) ppm: 7.88 (m, 1H, 9-Ph H-3); 7.47 (m, 2H, 9-Ph H-4 and 9-Ph H-5); 7.34 (t, 2H, *J* = 5.7, 2 × NHCO); 7.06 (m, 1H, 9-Ph H-6); 6.36 (s, 2H, Rhodamine H-4 and H-5); 6.16 (s, 2H, Rhodamine H-1 and H-8); 3.64 (m, 4H, 2 × $CONHCH_2CH_2NH_2$); 3.55 (br.s, 2H, 2 × ArNH); 3.21 (m, 8H, 2 × NH_2 and 2 × $ArNHCH_2$); 3.11 (m, 2H, CH_2NCOAr); 2.73 (m, 4H, 2 × CH_2NH_2); 2.55 (t, 4H, *J* = 6.1 Hz, 2 × CH_2CH_2CONH); 2.15 (m, 6H, $N(CH_2)_3$); 1.90 (s, 6H, 2 × $ArCH_3$); 0.93 (t, 6H, *J* = 7.2 Hz, 2 × CH_2CH_3). Elemental analysis: Calculated for $C_{38}H_{52}N_8O_4$ (MW 684.87) C 66.64, H 7.65, N 16.36%; Found C 66.38, H 7.71, N 16.24%.

*Synthesis of light-harvesting antenna (**7**)*

To a solution of 1.95 g (8 mM) of 4-nitro-1,8-naphthalic anhydride **5** in in 60 mL of boiling methanol, a solution of 2.74 (4 mM) of dendron **4** in 40 mL of methanol was added dropwise under stirring over a period of 2 h. The resulting solution was refluxed for 6 h. After cooling the brown precipitate was filtered off, treated with 50 mL of 5% aqueous sodium hydroxide to give after filtration and drying an intermediate **6**. To a solution of the intermediate **6** (3.18 g, 2.8 mM) in 50 mL of DMF, *N*,*N*-dimethylethylenediamine (1.5 mL, *d* = 0.807, 14 mM) was added portion wise at room temperature. After 48 h the resulting solution was poured into water. The precipitate was filtered off, washed with water and dried. Silica gel column chromatography using acetone as mobile phase afforded 2.76 g (57%) yellow solid of light-harvesting dendron **7**. FT-IR (KBr) cm^{-1}: 3346 (νNH); 2921 and 2885 (νCH); 1696 ($ν^{as}$N–C=O); 1648 ($ν^{s}$N–C=O); 1625, 1512 and 1449 (νAr=CH). ^1H NMR ($CDCl_3$-*d*, 250.13 MHz) ppm: 8.05 (d, 2H, *J* = 7.6 Hz, 2 × 1,8-naphthalimide H-5); 7.95 (d, 2H, *J* = 8.6 Hz, 2 × 1,8-naphthalimide H-2); 7.85 (m, 3H, 9-Ph H-3 and 2 × NHCO); 7.50 (d, 2H, *J* = 8.4 Hz, 2 × 1,8-naphthalimide H-7); 7.45 (m, 2H, 9-Ph H-4 and 9-Ph H-5); 7.07 (m, 1H, 9-Ph H-6); 6.87 (dd, 2H, *J* = 7.7 Hz, *J* = 8.4 Hz, 2 × 1,8-naphthalimide H-6); 6.37 (s, 2H, Rhodamine H-4 and H-5); 6.26 (d, 2H, *J* = 8.6 Hz, 2 × 1,8-naphthalimide H-3); 6.19 (s, 2H, Rhodamine H-1 and H-8); 5.78 (m, 2H, 2 × 1,8-naphthalimide ArNH); 4.11 (m, 4H, 2 × $(CO)_2NCH_2$); 3.84 (t, 4H, *J* = 5.6 Hz, 2 × 1,8-naphthalimide $ArNHCH_2$); 3.65 (m, 6H, 2 × $CONHCH_2$ and 2 × Rhodamine $ArNHCH_2$); 3.17 (m, 6H, 2 × Rhodamine $ArNHCH_2$ and CH_2NCOAr); 2.86 (t, 4H, *J* = 5.5 Hz, 2 × NCH_2); 2.52 (t, 4H, *J* = 6.2 Hz, 2 × CH_2CH_2CONH); 2.32 (s, 12H, 4 × NCH_3); 2.14 (m, 6H, $N(CH_2)_3$); 1.90 (s, 6H, 2 × Rhodamine $ArCH_3$); 1.33 (t, 6H, *J* = 7.1 Hz, 2 × Rhodamine CH_2CH_3). Calculated for $C_{70}H_{80}N_{12}O_8$ (MW 1217.46) C 69.06, H 6.62, N 13.81%; Found C 68.86, H 6.79, N 13.68%.

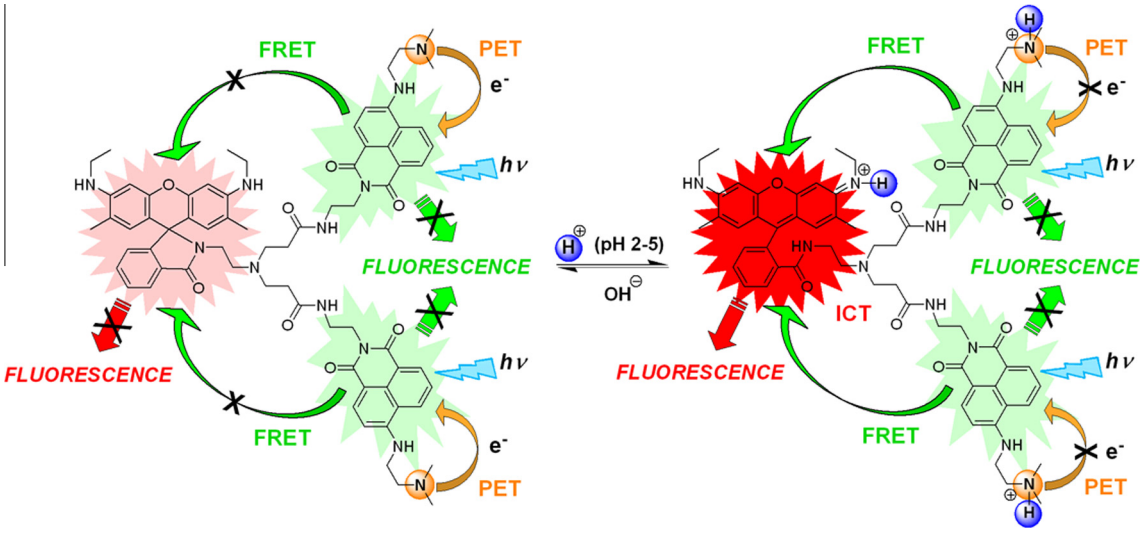
Results and discussion

Design and synthesis

Light harvesting PAMAM dendron **7** was configured as a FRET bichromophoric system, core and peripherally decorated with an ICT rhodamine acceptor and PET-based 1,8-naphthalimide donors, respectively. As the rhodamines are orange–red emitting fluorophores with maximal absorption at about $λ_A$ = 520–530 nm, where the emission of 4-alkylamino-1,8-naphthalimides is appeared, these two derivative groups are suitable fluorescence donor–acceptor pair for construction of wavelength-shifting bichromophoric systems [51].

To add "off–on" properties to the novel light harvesting dendron, peripheral 1,8-naphthalimide donors were decorated with *N*,*N*-dimethylethylenediamine receptor fragments. The 4-(*N*,*N*-dimethylamino)ethylamino-1,8-naphthalimides are well known "off–on" probes based on "fluorophore–spacer–receptor" format [24,39,52,53]. In this particular case, it could be predicted that a PET process would quench fluorescence of the periphery thus disabling the energy transfer in light-harvesting dendron **7** ("*off-state*" of the system). Binding the receptor with the analyte would increase its oxidation potential, and as such, thermodynamically disallow the electron transfer to the peripheral 1,8-naphthalimides. Consequently the energy transfer in light-harvesting dendron **7** would be "*switched on*" (Scheme 2).

Because of the pH sensitive character of rhodamine acceptor dye and "off–on" sensing properties of yellow–green emitting periphery, it could be expected that the fluorescence signal of the novel system would be a function of pH. Under neutral and alkaline conditions the rhodamine derivatives are in colorless spirolactam closed form and the PET based dialkylethylenediamino-1,8-naphthalimides are in their "*off-state*" [54]. Consequently the energy transfer from the peripheral 1,8-naphthalimes to the rhodamine core is not feasible and the emission of light-harvesting dendron **7** will be quenched (Scheme 2). In acid media (pH = 2–5) the rhodamine spirolactam ring is opened and the PET process in periphery is disallowed. Thus the energy of the peripheral 1,



Scheme 2. Photophysical behavior of antenna **7** as function of pH after excitation within a spectral region of maximal absorption of the peripheral fluorophores.

8-naphthalimides in antenna **7** will be transferred to the focal rhodamine and the systems will emit orange–red fluorescence signal.

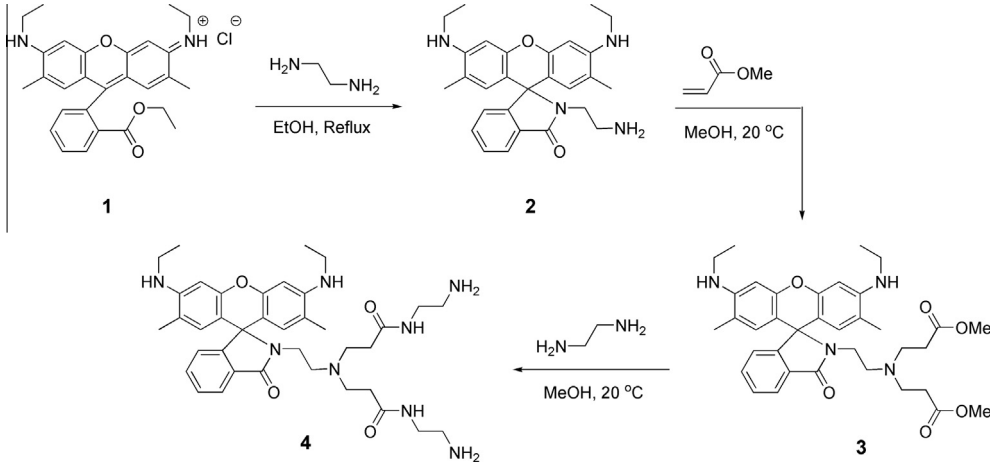
The novel light-harvesting antenna **7** was prepared in three basic steps: synthesis of amino-functionalized rhodamine core **2**, PAMAM dendronization of the rhodamine core to an amino-terminated dendron **4** and peripheral decoration of the latter with yellow–green emitting 4-(*N*,*N*-dimethylamino)ethylamino-1,8-naphthalimide units to the desired antenna.

The antenna core **2** was synthesized by reaction of Rhodamine 6G **1** with ethylenediamine under reflux in absolute ethyl alcohol. Then the rhodamine core **2** was subsequently converted into the PAMAM dendron **4** via divergent strategy involves initial Michael addition of **2** with methyl acrylate followed by exhaustive amidation of the resulting ester **3** with a large excess of ethylenediamine [21] (Scheme 3).

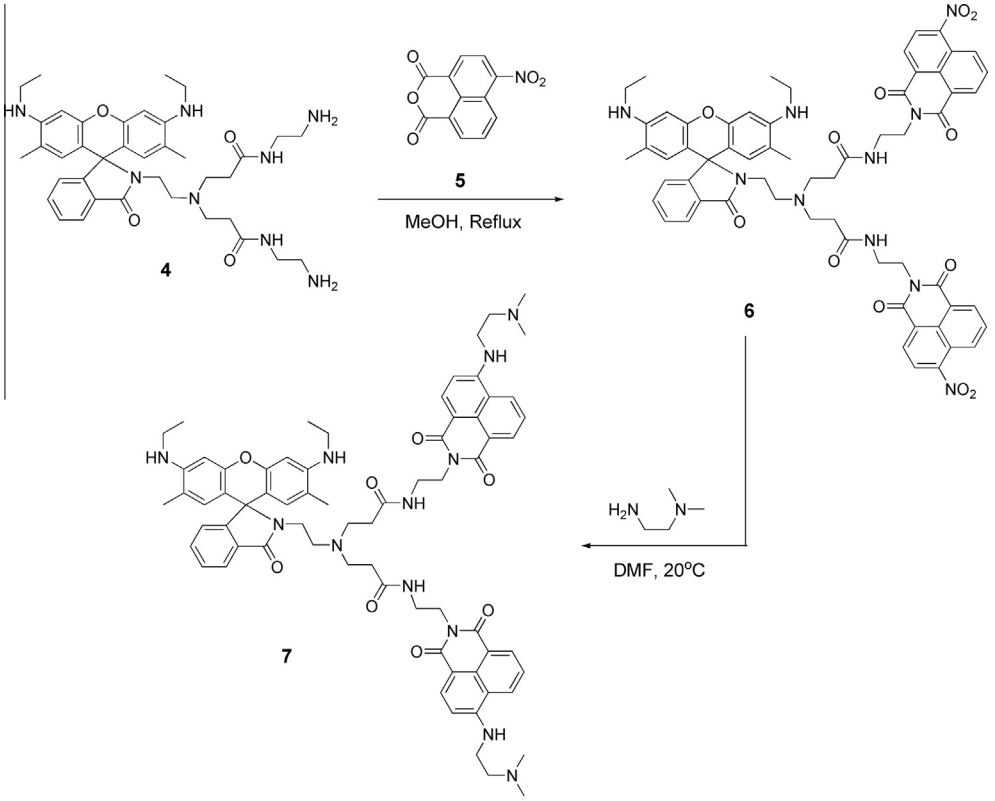
The target light harvesting antenna **7** was synthesized in two steps as shown in Scheme 4. First, the intermediate dendron **6** with 4-nitro-1,8-naphthalimide periphery was obtained by reaction of 4-nitro-1,8-naphthalic anhydride **5** and PAMAM dendron **4** under reflux in methanol solution. Finally, the yellow–green emitting periphery of the system was obtained after substitution of the nitro groups in the intermediate **6** with (*N*,*N*-dimethylamino)ethylamino moieties by reaction of **6** with *N*,*N*-dimethylethylenediamine in DMF at room temperature.

All of the synthesized compounds were characterized by their melting points and TLC retention values R_f (Table 1) and identified by conventional techniques – elemental analysis data, UV–Vis, fluorescence, FT-IR and ^{1}H NMR spectroscopy.

For instance, in the ^{1}H NMR ($CDCl_3$-*d*, 250.13 MHz) spectrum of antenna **7** (see Section 'Synthesis of light-harvesting antenna (**7**)') a resonance at 4.11 ppm was observed. This is characteristic for the methylene protons coupled to *N*-position of 1,8-naphthalimide moiety, which proves the presence of 1,8-naphthalimide units in the dendron periphery. Also the ^{1}H NMR spectrum of antenna **7** contains two resonances at 6.37 ppm and 6.19 ppm that are typical for the core Rhodamine 6G protons at position C-1, C-4, C-5 and C-8. The resonances at 4.11 ppm, 6.19 ppm and 6.37 ppm are in ratio 2:1:1 suggesting that in the novel dendron rhodamine core is surrounded by two 1,8-naphthalimide units. Furthermore, a resonance at 6.26 ppm is a typical characteristic for the proton in position C-3 of the yellow-green emitting 1,8-naphthalimide, substituted in position C-4 with an electron-donating *N*,*N*-dimetylethylenediamine group. This resonance is rather different from the corresponding value for a non-substituted 4-nitro-1,8-naphthalimide moieties (8.35–8.70 ppm) [21,55], which is a solid evidence that the peripheral 4-nitro-1,8-naphthalimides in the intermediate dendron **6** were completely converted into yellow–green emitting donor periphery. Moreover the ^{1}H NMR spectrum contains all



Scheme 3. Synthesis of rhodamine core **2** and intermediate amino-terminated dendron **4**.



Scheme 4. Synthesis of PAMAM light-harvesting antenna **7**.

Table 1
Yields, melting points and retention factors for intermediates **2**–**4** and target antenna **7**.

Compound	Yield (%)	M.p. (°C)	R_f
2	85	>250	0.46[a]
3	96	133–135	0.68[b]
4	94	89–91	0.29[c]
7	57	155–157	0.49[c]

[a] TLC: chloroform/ethylacetate/ethanol = (1:1:1).
[b] TLC: toluene/ethanol = (2:1).
[c] TLC: *n*-propanol/ammonium hydroxide = (1:1).

requisite peaks for rhodamine and 1,8-naphthalimide moieties as well as peaks in the range of 2.2–4.0 ppm, attributed to the protons in the peripheral (*N*,*N*-dimethylamino)ethylamino moieties.

Photophysical characterization of antenna **7**

The basic photophysical characteristics of antenna **7** and intermediate dendron **4** were determined in water/DMF (4:1, v/v) solution. Under these conditions the rhodamine moiety adopts a closed, non-fluorescent spirolactam form. At *ca.* pH 2 the spirolactam ring of rhodamine is opened, which results in new absorption and emission (rhodamine) bands with well pronounced maxima at 532–534 nm and 560 nm, respectively. The recorded photophysical characteristics for the rhodamine core in intermediate **4** and antenna **7** were common for the Rhodamine 6G derivates [56] (Table 2). The presented data show that the incorporation of Rhodamine 6G in the light-harvesting antenna does not affect the energy and the shape of the dye absorption and emission bands.

In water/DMF (4:1, v/v) light-harvesting antenna **7** (pH 6.5, spirolactam closed form) showed absorption band in range of about 370–510 nm, which is attributed to an internal charge transfer process in the 1,8-naphthalimide chromophores. After acidification to *ca.* pH 2 where the rhodamine spirolactam is opened, the absorption spectrum of light harvesting system **7** showed two bands (Table 2) corresponding to the absorption location of the peripheral 1,8-naphthalimide chromophores (λ_A = 442 nm) and the focal rhodamine (λ_A = 534 nm). The calculated molar extinction coefficient value of the peripheral absorption of the light harvesting antenna **7** is about 2 times higher than the usual for 4-amino-1,8-naphthalimide derivatives [54], suggesting no ground state interaction between the peripheral 1,8-naphthalimide units.

The fluorescence spectrum of light-harvesting antenna **7** in water/DMF (4:1, v/v) solution, recorded at pH 6.5 after excitation within the spectral region of maximal absorption of the donor fluorophore (λ_{ex} = 420 nm), showed weak emission centered at 533 nm, corresponding to the emission band of the donor 1,8-naphthalimide fragments in the donor–acceptor system. Under these conditions the peripheral 1,8-naphthalimides are in "*off-state*" due to the PET process from the tertiary amine receptors. When the fluorescence spectrum was recorded at *ca.* pH 2, the observed emission was shifted to 560 nm, which should be attributed to the energy transfer from the donor 1,8-naphthalimides to the ring-opened (fluorescence) form of the rhodamine acceptor.

Table 2
Photophysical characteristics of compounds **4** (λ_{ex} = 510 nm) and **7** (λ_{ex} = 420 nm) in water/DMF (4:1, v/v).

Compound	λ_A (nm)	ε (l mol^{-1} cm^{-1})	λ_F (nm)	ν_A–ν_F (cm^{-1})	Φ_F
4	532[a]	43,000[a]	560[a]	940[a]	0.55[a]
7	442[a,b]	22,000[a,b]	533[b]	3860[b]	0.02[b]
	534[a]	42,000[a]	560[a]	870[a]	0.40[a]

[a] Photophysical data recorded at *ca.* pH 2 to a avoid rhodamine spirolactam closed (non-fluorescent) form.
[b] Photophysical data recorded at *ca.* pH 6.5.

The Stoke's shift (ν_A–ν_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 shifts (cm^{-1}) were calculated by Eq. (1).

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

The Stoke's shift values for dendron **4** (940 cm^{-1}) and antenna **7** (870 cm^{-1}) observed after excitation at *ca.* pH 2 are typical for Rhodamine 6G [56] and do not indicate remarkable changes in the core fluorophore excited state due to the incorporation in the dendritic light-harvesting system. Also, after excitation at 420 nm (1,8-naphthalimide absorption region) and pH 6.5 (spirolactam closed form) the light-harvesting system **7** shows Stoke's shift value 3860 cm^{-1} that is usual for 1,8-naphthalimide fluorophores [54].

The ability of the molecules to emit the absorbed light energy is characterized quantitatively by the fluorescence quantum yield (Φ_F). The quantum yields of fluorescence were calculated using Rhodamine 6G (Φ_F = 0.95 in ethanol [49]) or Coumarin 6 (Φ_F = 0.78 in ethanol [50]) as standards according to Eq. (2), where A_{ref}, S_{ref}, n_{ref} and A_{sample} S_{sample}, n_{sample} represent the absorbance at the exited wavelength, the integrated emission band area and the solvent refractive index of the standard and the sample, respectively.

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

As can be seen (Table 2), the fluorescence quantum yield of rhodamine core in dendron **4** is higher as compared to the quantum yield of focal rhodamine in antenna **7**. Most probably this could be attributed to the higher hydrophobicity of light harvesting dendron **7** in respect to the amino-terminated dendron **4** which results in stronger self-quenching effect in the presence of water due to aggregation of the hydrophobic peripheral fluorophores.

Influence of pH on the photophysical properties of antenna **7**

The light-harvesting system under study was designed as a molecular fluorescence probe for determination of pH changes over a wider pH scale. This was the reason to investigate its photophysical behavior in water/DMF (4:1, v/v) solution at different pH values. It was found that the presence of alkali metal ions do not affect the emission and absorption properties of antenna **7**. In the presence of K^+ and Na^+ only minor fluorescence quenching was observed which is attributed to the increased ionic strength. That is why the pH was adjusted with aqueous NaOH. Also, for a constant ionic strength the experiments were performed in the presence of NaCl.

In order to receive a more complete comparative picture for the influence of the PAMAM bone to the focal rhodamine unit at different pHs, intermediate dendron **4** was involved in the present study as a reference compound. As can be seen, the decrease of pH results in increase of the absorbance at 532 nm due to the ring opening reaction of rhodamine core (Fig. 1).

The changes of the absorption maximum of dendron **4** at 532 nm as a function of pH in water/DMF (4:1 v/v) are plotted in the inset of Fig. 1. Taking the part of the graph located between pH 2 and 6, the calculated by Eq. (3) [57] pK_a value of PAMAM dendron **4** was 4.2.

$$\log[(A_{max} - A)/(A - A_{min})] = pH - pK_a \quad (3)$$

The absorption spectrum of light-harvesting antenna **7** does not show significant pH-dependent changes in a pH window 5–8 as the 1,8-naphthalimide fluorophores do not affect their ICT excited states (Fig. 2). The decrease of pH from 5 to 2 results in a novel

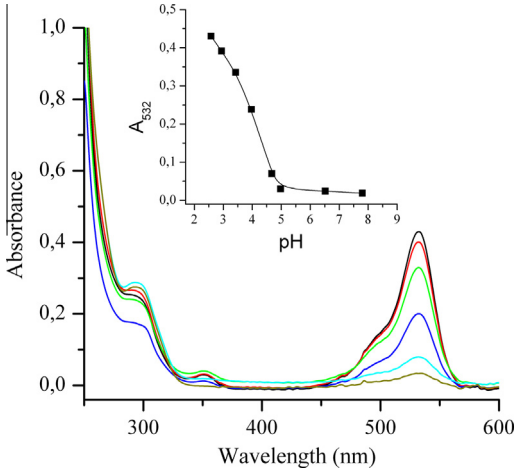


Fig. 1. Absorption changes of dendron **4** as a function of pH in water/DMF (4:1, v/v) with addition of NaCl (0.01 M) for constant ionic strength. Inset: Titration plot of dendron **4** in a pH range of *ca.* 8–2.

band corresponding to the absorption of the focal rhodamine in the examined antenna. From the absorption changes at 534 nm the titration curve of the system was obtained. The calculated pK_a value of 4.4 for antenna **7** using Eq. (3) is similar to that for PAMAM dendron **4**.

The fluorescent spectrum of dendron **4** excited at 510 nm was also recorded in water/DMF (4:1, v/v) solution at different pH values. In alkaline solution the rhodamine core in dendron **4** is in spirolactam closed form and does not emit light. However upon acidification the emission signal in a range between 500 nm and 700 nm was gradually increased as this is demonstrated in Fig. 3.

Analysis of the fluorescence changes at 560 nm (Inset of Fig. 3) according to Eq. (4) [58] gives pK_a value of 4.1 which is practically the same as the pK_a value, calculated from the absorption changes, and is attributed to the rhodamine spirolactam opening reaction.

$$\log[(I_{Fmax} - I_F)/(I_F - I_{Fmin})] = pH - pK_a \quad (4)$$

In alkaline and neutral media, after excitation at 420 nm (within the spectral region of maximal absorption of the donor fluorophores), antenna **7** shows an emission band of low intensity with maximum at 533 nm. Under these conditions the focal accep-

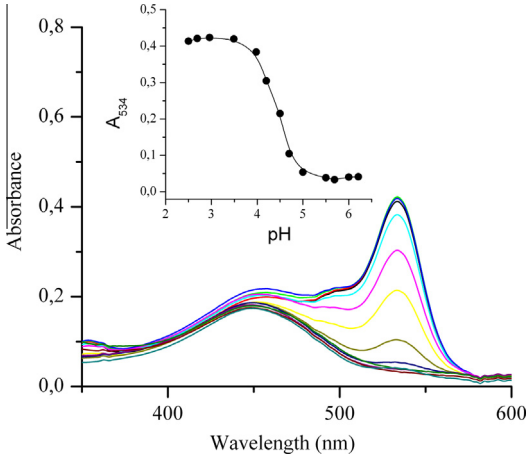


Fig. 2. Absorption changes of antenna **7** as a function of pH in water/DMF (4:1, v/v) with addition of NaCl (0.01 M) for constant ionic strength. Inset: Titration plot of antenna **7** in a pH range of *ca.* 7–2.

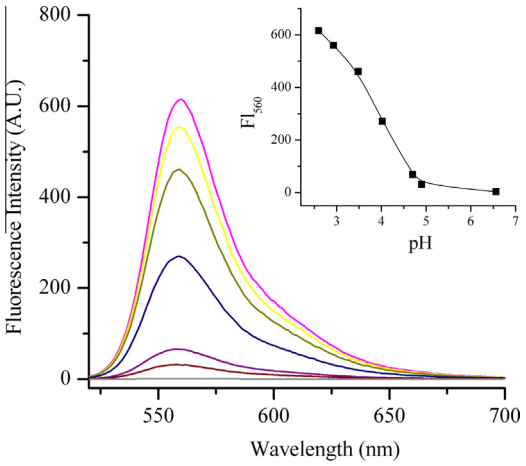


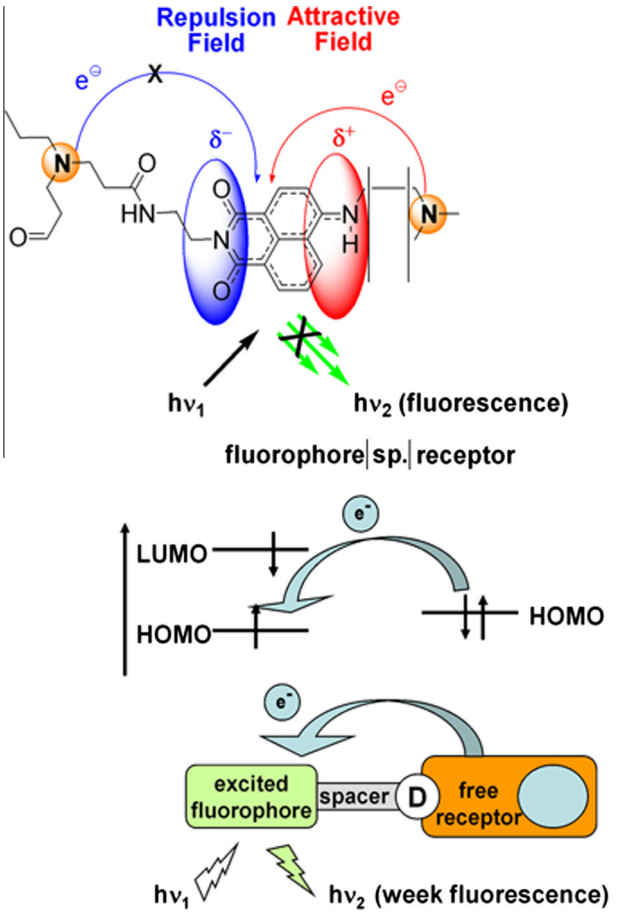
Fig. 3. Fluorescent changes of dendron **4** as a function of pH in water/DMF (4:1, v/v) with addition of NaCl (0.01 M) for constant ionic strength (λ_{ex} = 510 nm). Inset: Titration plot of dendron **6** in a pH range of *ca.* 8–2.

tor dye is in spirolactam form and energy transfer from the peripheral donor fluorophores to the core is not faceable. At the same time, the PET process in the donating 1,8-naphthalimides quenches the peripheral fluorescence and as a consequence antenna **7** shows only weak emission in the typical for the 4-amino-1,8-naphthalimides yellow–green region.

It should be noted that only receptors, directly attached to the 1,8-naphthalimide 4-amino moiety ("lower" moiety) are capable of quenching the fluorophore excited state [59]. The 4-amino-1,8-naphthalimide fluorophores are "push–pull" π electron systems with strong internal charge transfer (ICT) in the lowest excited singlet state and considerable dipole character (positive pole at the 4-amino terminus). A large dipole moment gives rise to a strong photogenerated electric field that can, depending on its magnitude and sign, inhibit or accelerate transition of the electron in the 1,8-naphthalimide chromophore. Thus the quenching PET process is observed only if the electron leaving the unprotonated amine receptor can enter the space of the 4-amino-1,8-naphthalimide fluorophore across C-4 position with its attractive electric field (Scheme 5). The corresponding PET path from the receptor in *N*-position is just as feasible thermodynamically but requires the electron to enter the fluorophore across the imide moiety with its repulsive electric field and is not observed.

In water/DMF (4:1, v/v), the peripheral 4-alkylamino-1,8-naphthalimides [54] and rhodamine core [20] showed pH sensing properties in a very similar pH window (2–5). Upon acidification from pH 6 to pH 2 the rhodamine spirolactam form became opened and the peripheral amine receptors were protonated thus disallowing the PET quenching process in the donor 1,8-naphthalimides. Under these conditions the absorbed by the peripheral 1,8-naphthalimides energy in antennae **7** (λ_{ex} = 420 nm) is transferred to the focal Rhodamine and the system emits red–orange fluorescence. This results in remarkable fluorescence intensity enhancement (FE) in the rhodamine emission region at 560 nm (Fig. 4A). The FE = I/I_0, calculated using minimal (I_0) and maximal (I) fluorescence intensity, recorded in the examined pH interval was almost 30 times (FE = 29.7).

On the basis of the fluorescence changes of antenna **7** a reitiometric titration curve as a function of pH was obtained using the ratio between 560 and 520 nm (Inset of Fig. 4A). Analysis of the ratiometric titration curve of antenna **7** after excitation at 420 nm according to Eq. (4) gives the pK_a value of 4.3. Similar results (pK_a = 4.4) were obtained after direct core excitation at 510 nm (Fig. 4B, Inset of Fig. 4B) and from the absorption changes (Inset of Fig. 2) of antenna **7**, suggesting that only the spirolactam-opening



Scheme 5. Feasible photoinduced electron transfer in the 1,8-naphthalimide periphery of antenna **7**.

reaction of rhodamine core is responsible for the pH sensing properties of the system.

Influence of metal cations on the fluorescence intensity of antenna ***7***

With regard to its potential application as a fluorescent probe for cations recognition, the signaling properties of light-harvesting dendron **7** were investigated in the presence of different metal cations (Co^{2+}, Cu^{2+}, Fe^{3+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Hg^{2+} and Ag^{+}). Experiments were performed in buffered with 0.001 M HEPES (pH 7.3) water/DMF (4:1, v/v) solutions. The effect of the metal cations was observed using two methods. The first one was carried out by adding of the metal stock solutions to different buffered solutions of the examined dendron **7**. The second one was performed by addition of buffer solutions to solutions of dendron **7** in the presence of the metal cation. In both cases a very similar effect was observed.

The addition of the above metal ion solutions (up to 1×10^{-4} M concentration) to the buffered solution of antenna **7** (1×10^{-5} M) caused minor quenching in the fluorescence intensity (below 10%) of the compound. Further increase of the metal ion concentration (up to 1×10^{-3} M) strongly quenches the emission of the light-harvesting system **7**. In the presence of most of the tested metal ions the quenching effect was 15–20% and in some cases higher than 20% (Fig. 5).

The results obtained suggest that representative metal ions are not able to bind both the peripheral amine and core spirolactam receptors of antenna **7** under these conditions. The quenching effect of metal cations could be easily rationalized as follow: (i)

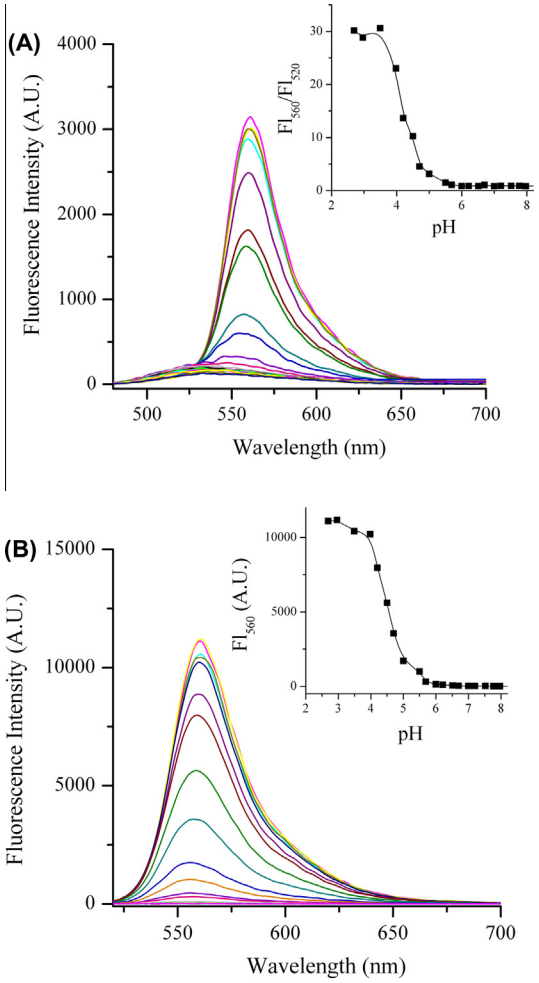


Fig. 4. Fluorescent changes of antenna **7** as a function of pH in water/DMF (4:1, v/v) with addition of NaCl (0.01 M) for constant ionic strength, excited at 420 nm (**A**) and 510 nm (**B**). Inset: Titration plots of antenna **7** excited at 420 nm (**A**) and 510 nm (**B**) in a pH range of *ca.* 8–2.

Metal cations cause paramagnetic fluorescence quenching due to complex formation in the dendrimer bone resulting in energy transfer toward the peripheral 1,8-naphthalimide units [60]; (ii) At pH 7.3 the tested metal ions produce insoluble hydroxides

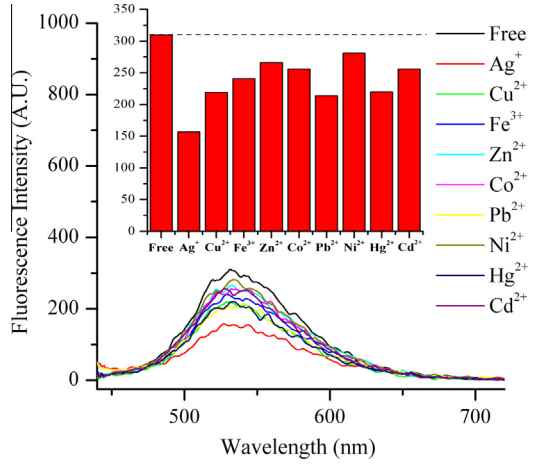


Fig. 5. Effect of metal cations at concentration 1×10^{-4} mol L^{-1} on the fluorescence intensity of light-harvesting antenna **7** ($C = 1 \times 10^{-5}$ mol L^{-1}) in 1 mM HEPES (pH 7.3).

which alters the optical properties of the sample and thereby quenches the fluorophore emission.

Based on the performed experiments and the results obtained it can be generalized that only protons are able to enhance the fluorescent intensity at 560 nm of light-harvesting dendron **7** which suggest the excellent sensing selectivity of the novel compound toward protons. The undesired fluorescence quenching in the presence of different metal cations can be easily avoided by using pH ratiometric analysis because the metal ions do not affect the ratio between the fluorescence intensity at 560 nm and 520 nm. The ratiometric pH sensitivity and excellent pH selectivity of the novel wavelength-shifting bichromophoric system in aqueous medium may be beneficially for monitoring pH variations in complex samples.

Conclusions

A novel PAMAM wavelength-shifting bichromophoric system, core and peripherally decorated with Rhodamine 6G and 1,8-naphthalimide fluorophores, respectively, was synthesized based on a divergent approach. The system was designed as a light-harvesting antenna capable of absorbing light by its periphery and efficiently transferring the energy to a single acceptor dye in the focal point of the molecule. The yellow–green emitting periphery of the antenna was designed on the "fluorophore–spacer–receptor" format able to act as a fluorescence PET-based pH probe. In alkaline and neutral media, after excitation within the spectral region of maximal absorption of the peripheral 1,8-naphthalimides, the synthesized antenna showed only weak peripheral fluorescence, while upon acidification to pH 2 fluorescence intensity of the core was enhanced almost 30 times (FE = 29.7). Novel bichromophoric system also showed excellent selectivity toward protons over representative metal ions. Thus the distinguishing FRET features of the light-harvesting antennae were successfully combined with the properties of classical PET and ring-opening ICT molecular systems. The combination of the three effects make the antenna able to act as a highly selective and pH sensitive ratiometric fluorescence probe in aqueous medium. This indicates that the system could be useful as a practical tool for analysis of complex environmental samples and probably for biological studies.

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