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Cellular imaging using BODIPY-, pyrene- and phthalocyanine-based conjugates

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ABSTRACT

Fluorescent Probes aimed at absorbing in the blue/green region of the spectrum and emitting in the green/red have been synthesized (as the form of dyads-pentads), studied by spectrofluorimetry, and used for cellular imaging. The synthesis of phthalocyanine-pyrene **1** was achieved by cyclotetramerization of pyrenyldicyanobenzene, whereas phthalocyanine-BODIPY **2c** was synthesized by Sonogashira coupling between tetraiodophthalocyanine and meso-alkynylBODIPY. The standard four-steps BODIPY synthesis was applied to the BODIPY-pyrene dyad **3** starting from pyrenecarbaldehyde and dimethylpyrrole. ^{1}H, ^{13}C, ^{19}F, ^{11}BNMR, ICP, MS, and UV/Vis spectroscopic analyses demonstrated that **2c** is a mixture of BODIPY-Pc conjugates corresponding to an average ratio of 2.5 BODIPY per Pc unit, where its bis, tris, tetrakis components could not be separated. Fluorescence emission studies (μM concentration in THF) showed that the design of the probes allowed excitation of their antenna (pyrene, BODIPY) in the blue/green region of the spectrum, and subsequent transfer to the acceptor platform (BODIPY, phthalocyanine) followed by its emission in the green/red (with up to 140–350 nm overall Stokes shifts). The fluorescent probes were used for cellular imaging of B16F10 melanoma cells upon solubilization in 1% DMSO containing RPMI or upon encapsulation in liposomes (injection method). Probes were used at 1–10 μM concentrations, cells were fixed with methanol and imaged by biphoton and/or confocal microscopy, showing that probes could achieve the staining of cells membranes and not the nucleus.

1. Introduction

In biphoton imaging, deeper penetration of light in tissues is possible compared to monophoton excitation.[1] Conjugating a blue-absorbing dye to another NIR-emitting-dye will make emission in the NIR possible provided that either FRET or through bond energy transfers (TBET) occur. Hence, such a strategy is a twofold approach that will minimize the autofluorescence on the way in (biphoton) and way out (TBET followed by emission in the NIR).

The rational of our study was to develop dyads/pentads of fluorophores in order to harvest light on a large window in biphoton microscopy. We selected three well-known classes of fluorophores that span the optical window: Pyrene (380 nm, blue), BODIPY (500 nm, green), and Phthalocyanine (Pc, 680 nm, near-IR)[2] (which are also known as robust and efficient photosensitizers in photodynamic therapy[3a,b]). Hence, we were interested in fluorophore conjugates that could be used in cellular imaging upon transferring light from 300 nm to 700 nm (as in Pyrene-Pc **1a** reported by Ozcesmeci et al.[4]), 500 nm to 700 nm (as in BODIPY-Pc **2c**) and 300 nm to 500 nm (as in Pyrene-BODIPY **3a** reported by Goze et al.[5]). Some of these fluorescent molecular assemblies have been previously reported but have not been used as probes for cellular imaging. However, the design of BODIPY-Pc conjugates **2a** and **2b** reported by Göl et al.[6a] and Osati et al.,[6b] respectively, are slightly different than **2c** depicted in Fig. 1. Göl et al. BODIPY-Pc conjugate **2b** has either one phenyl ring or a —CH_2O— between the BODIPY and the alkyne, whereas our system **2c** provides a shorter connection between Pc and BODIPY that are separated with only one alkynyl group. The rationale for such an optimized

Abbreviations: FRET, Förster resonance energy transfer; TBET, through-bond energy transfer; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; Pc, phthalocyanine; BODIPY, BOron DIPYromethene; NMR, nuclear magnetic resonance; UV–Vis, UltraViolet–visible; DMSO, dimethyl sulfoxide; DCM, dichloromethane; $CDCl_3$, deuterated chloroform; THF, tetrahydrofuran; PBS, phosphate buffered saline; RPMI, ≪Roswell Park Memorial Institute≫ medium; TMS, TriMethylSilyl; ICP-AES, inductively coupled plasma – atomic emission spectrometry; LC-MS, liquid chromatography – mass spectrometry; HPLC, high performance liquid chromatography; MALDI-TOF, matrix-assisted laser desorption/ionization – time of flight; ESI-MS, electrospray ionization mass spectrum.

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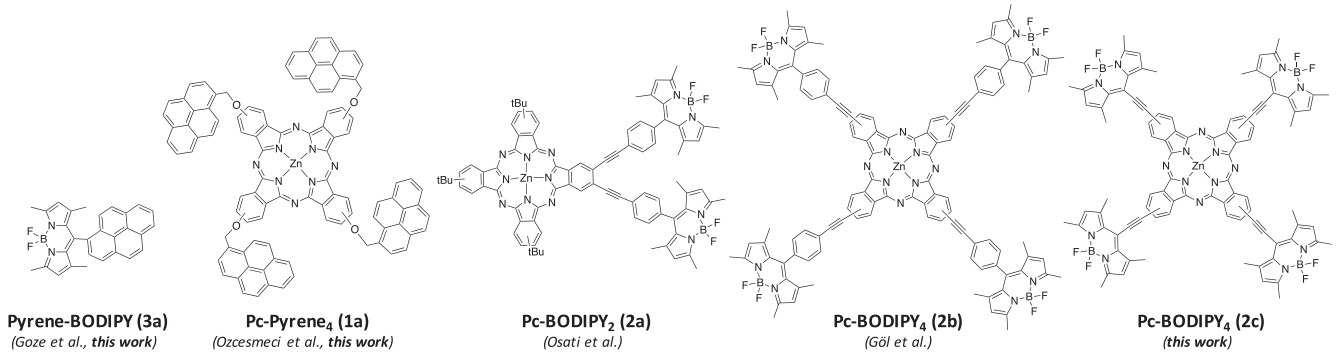

Fig. 1. Synthetic dyads/triads/pentads described in the literature (**1**, **2a**, **3**) and reported in this work (**2c**), that span the 300–700 nm spectrum region.

design was to hope for a better transfer between the two antennas within the dyad by increasing the conjugation between the two antennas. At this stage of the study the yields of transfers were not measured.[7]

2. Materials and methods

2.1. Materials

Chemicals were used as provided.

2.2. Measurements and instrumentations

LC–MS mass spectrometry analyses, Analytical and reverse phase HPLC were performed on a Ultimate 3000 Thermo DIONEX apparatus. MALDI-TOF mass spectrometry analyses were performed on an Ultraflex II LRF 2000 apparatus, using dithranol and DHB as a matrix. ICP analyses were performed on a ICP-AES iCAP 7400 double visée Thermo apparatus. Low-resolution ESI analyses were carried out on an Amazon SL (BRUKER) mass spectrometer. High-resolution ESI analyses were performed on a LTQ Orbitrap XL (THERMO) apparatus. ^{1}H NMR, ^{19}F NMR and ^{11}B NMR analyses were performed on a 300 MHz Bruker Avance III NanoBay NMR apparatus. UV/Vis absorption spectra were measured using a UV–Visible AGILENT CARY 50 spectrophotometer. Fluorescence measurements, excitation and emission spectra were recorded on a Fluorolog Jobin Yvon Horiba Xenon Lamp spectrometer. Biphotonic images were collected on a Nikon A1-MP scanning microscope (Nikon, Japan). Imaging was carried out with a ×25 Apo LWD objective (NA: 1.1, Water Immersion, Nikon, Japan) at a scanning speed of 0.5 frame per second. An IR laser (Chameleon, Coherent) was used to provide a 780 nm excitation. Fluorescence emission was collected on four detection channels (channel 1 (blue): FF01-492/SP-25 filter [400–492 nm]; channel 2 (green): FF03-525/50-25 filter [500–550 nm]; channel 3 (orange): FF01-575/25-25 filter [563–588 nm]; channel 4 (red) FF01-629/56-25 filter [601–657 nm], Semrock). Confocal images were collected using a Nikon C1Si Eclipse TE 2000 confocal microscope (Nikon, Japan). Imaging was carried out with a ×100 PlanApo objective (NA: 1.4, oil, Nikon, Japan). Two laser diodes were used as light sources, which delivered 488 and 561 nm wavelength light. Fluorescence emission was collected by a spectral detector, using collection bands of [489–648] nm with 488 nm excitation and [566–721] nm for 561 nm excitation.

2.3. Methods

2.3.1. Syntheses of compounds ***1–9***

*2.3.1.1. [2,9(10),16(17),23(24)-Tetramethoxypyrenylphthalocyanina to]zinc(II) (**1**).* 4-(Pyren-1-ylmethoxy)-phthalonitrile (**5**) (70 mg, 0.19 mmol), zinc acetate (10 mg, 0.048 mmol), 1,8-diazabicyclo {5.4.0}undec-7-ene (21 mg, 0.19 mmol) and pentanol (10 mL) were placed in a round bottom flask. The mixture was heated to reflux at 140 °C for 22 h. The reaction mixture was subsequently allowed to come to room temperature, and then an ice bath was added to precipitate the product down. The precipitate was filtrated on a Büchner, rinsed with water, methanol, ethanol and acetone. Yield = 17%.

^{1}H NMR (300 MHz, DMSO d_6, 300 K): δ (ppm) = 6.2 (s, 8H); 7.8–8.7 (m, 48H). MS (MALDI-TOF): m/z = 1497.89 $[M+H]^+$, 1283.83 $[M-(CH_2\text{-pyr})+H]^{\cdot}$, 1069.78 $[M-(CH_2\text{-pyr})_2+2H]^{\cdot}$, 855.53 $[M-(CH_2\text{-pyr})_3+3H]^{\cdot}$, 641.13 $[M-(CH_2\text{-pyr})_4+4H]^{\cdot}$. (Calcd for $C_{100}H_{57}N_8O_4Zn$: 1497.37 (exact mass); 1498.95). UV–Vis (THF): λ_{max} (nm) = 329, 344, 611, 680.

*2.3.1.2. Phthalocyanine-Bodipy conjugates (**2c**) (Sonogashira coupling).* The design and synthetic approaches were inspired from previously reported molecules. Refs. 4,6,8. Pc-I_4 (**7**) (100 mg, 0.092 mmol), the alkyne-BODIPY (**6**) (100 mg, 0.36 mmol) and copper iodide (35 mg, 0.18 mmol) were put in a two-necked round bottom flask. Dry tetrahydrofuran (10 mL) and triethylamine (5 mL) were added, and the resulting mixture was degased upon three series of freeze-pumpthaw-nitrogen refill cycles. Tetrakis-(triphenylphosphine)-palladium (35 mg, 0.03 mmol) was added, and the mixture was stirred for 18 h. Ammonium chloride was added to quench the reaction, the organic phase was extracted with dichloromethane, washed with water and with a saturated solution of ammonium chloride, and the solvents were removed under reduced pressure. The resulting powder was washed with methanol and dichloromethane. Yield = 60%.

^{1}H NMR (300 MHz, DMSO d_6, 300 K): δ (ppm) = 2.5–3 (broad s, H from BODIPY methyl); 6.04 (broad s, H from BODIPY core) (hard to define the exact number of proton, no well-define peak), 7.0–8.5 (broad signal, H from Pc) ^{19}F NMR (280 MHz, d DMSO, 300 K): δ (ppm) = −146 ppm (not well-defined peak). ^{11}B NMR (128 MHz, d DMSO, 300 K): δ (ppm) = 0.524 (not well-defined peak). MALDI-TOF: m/z = 1224.85 $[M_{mono}+H]^+$; 1214.82 $[M_{mono}-9]^+$, 1369.06 $[M_{bis}+H]^+$; 1349.06 $[M-F]^+$; 1359.03 $[M_{bis}-9]^+$; 1513.26 $[M_{tris}-9]^+$; 1493.26 $[M-F]^+$; 1503.23 $[M_{tris}-9]^+$; 1647.42 $[Mtetra-9]^+$ (calcd for $C_{92}H_{68}B_4F_8N_{16}Zn$: 1656.53 (exact mass); Mono 1223.88; Bis 1368.10; Tris 1512.32; Tetra 1656.23). ICP-AES: Zinc $_{theorical}$ = 3.94%, Zinc $_{exp}$ = 3.83%, Boron $_{theorical}$ = 2.61%, Boron $_{exp}$ = 1.63%. UV–Vis (DCM): λ_{max} (nm) = 374, 567, 697.

*2.3.1.3. Pyrenyl-BODIPY (**3**).* Pyrenecarboxaldehyde (230 mg, 1 mmol) and dimethylpyrrole (0.215 mL, 2 mmol) were put in a round bottom flask then 30 mL of dry dichloromethane was added under nitrogen atmosphere, followed by one drop of trifluoroacetic acid. The solution became red, and the mixture was stirred for 16 h under nitrogen atmosphere. p-Chloranil (250 mg, 1 mmol) was added and the solution was stirred for 30 min under nitrogen atmosphere. Triethylamine (3 mL, 22 mmol) and boron trifluoride diethyletherate