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Synthesis and characterization of BODIPY fluorescent dyes for selective staining of intracellular organelles

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INTRODUCTION

Fluorescence microscopy is a powerful technique mostly used in biology for observation of different processes in cells [1]. It relies on the use of fluorescent dyes, which are expected to show specific fluorescent response depending on their localization [2]. Although a large number of different dyes have been discovered to date, there is contant demand for new fluorescent dyes.

BODIPY dyes are derivatives of 4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene, and were synthesized for the first time by Treibs and Kreuzer [3]. Because of the excellent spectroscopic and photochemical properties, they are widely used in material science, fluorescence sensing, anti-cancer phototherapy, as fluorescent building blocks, *etc*. BODIPY dyes have been structurally modified to specifically localize into specific organelles [4].

Herein we present synthetic methodologies for modification of BODIPY dye for selective localization in the lysosome, endoplasmic reticulum, and mitochondria.

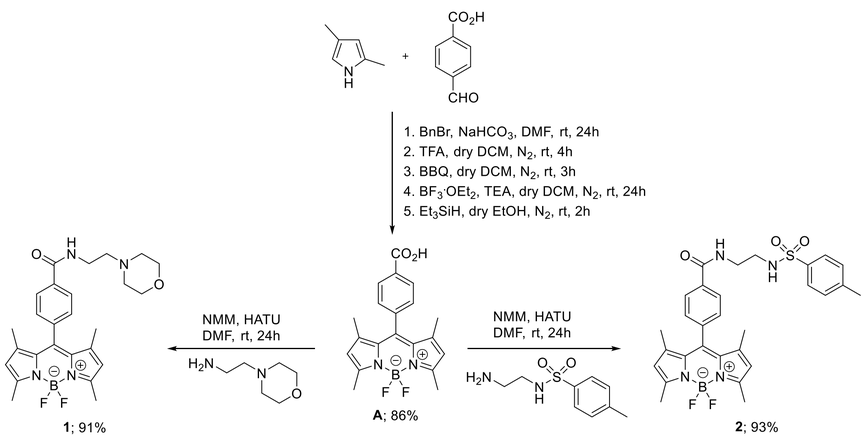
RESULTS [5]

Synthesis

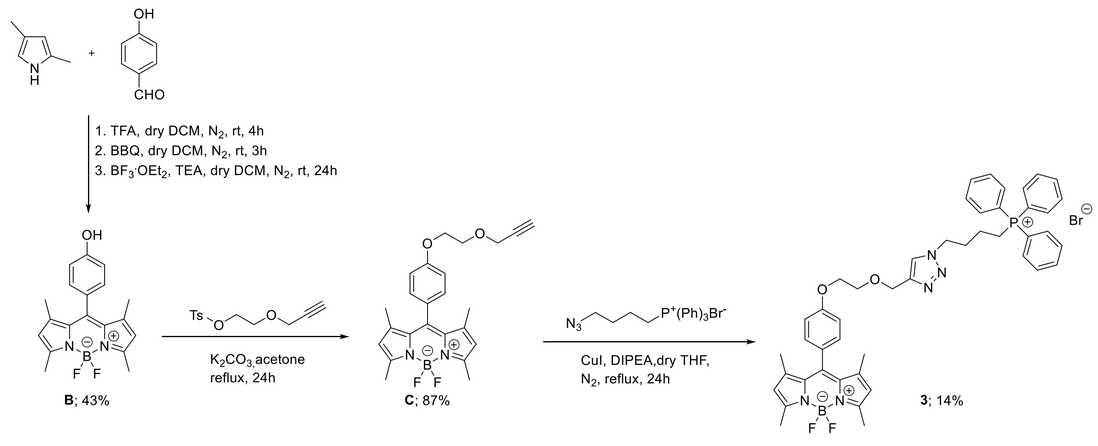
of BODIPY carboxylic acid **A** was carried out through several steps from 2,4-dimethylpyrrole and *p*-formylbenzoic acid, and product was isolated in excellent yield of 86 % (Scheme 1). Compounds **1** and **2** were synthesized from **A** by use of peptide coupling conditions.

BODIPY alkyne **C** was synthesized in excellent yield of 87 %, and was used in click reaction with azide triphenylphosphonium salt, for the preparation of compound **3** (Scheme 2).

Enzimatic stability of **1** and **2** towards lipase activity was determined using lipase B candida antartica and triacylglycerol lipase. Compounds confirmed their stability under enzymatic conditions during 24h.



Scheme 1. Synthesis of BODIPY compounds **1** and **2** by peptide coupling protocol.



Scheme 2. Synthesis of BODIPY compound **3** by click chemistry.

Fluorescence microscopy

was used to determine localization of BODIPY dyes in the organelles of interest. BODIPY compounds were tested on immortal and live HeLa cells.

Localization of BODIPY **1 - 3** was compared with Lysotracker RED, endoplasmic reticulum tracker, and mitotracker RED, respectively. All three BODIPY dyes showed strong localization in the lysosome, endoplasmic reticulum, and mitochondria, respectively (Figure 1). The Pearson correlation coefficient was calculated, and the values were between 0.90 – 0.94.

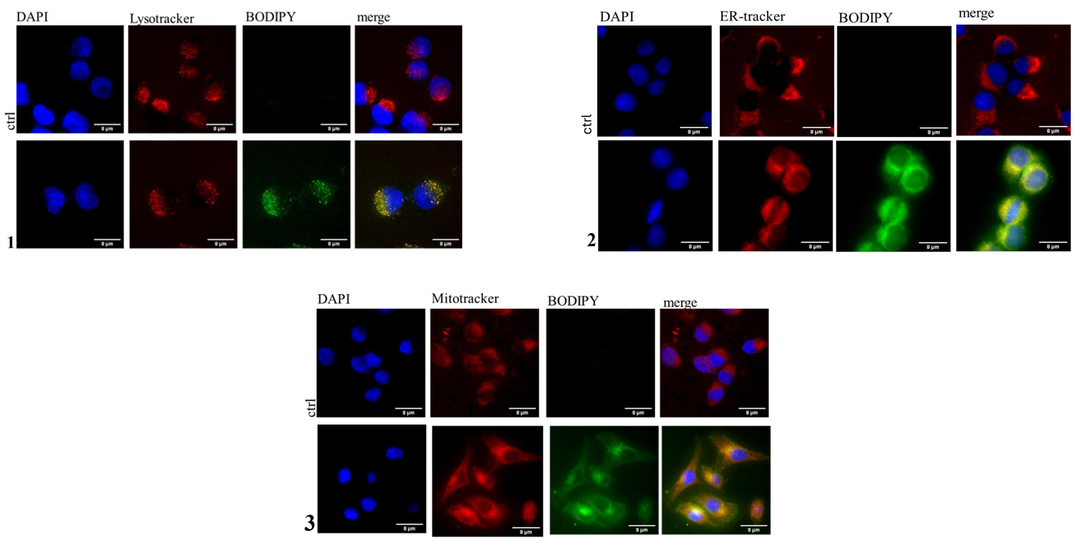


Figure 1. Fluorescence microscopy of HeLa cells incubated with BODIPY derivatives **1**, **2** and **3** respectively.

Spectral and photophysical properties

of **1** – **3** were investigated in polar protic ($CH_3CN:H_2O$ = 1:10), polar aprotic (CH_3CN), and nonpolar solvents (toluene and CH_2Cl_2). BODIPY dyes **1** – **3** showed maximum of absorption spectra at ≈ 497 – 502 nm (Figure 2a, 2b), and molar absorption coefficients 35000 – 100000. The absorption spectra showed negligible solvatochromic shift of 5 nm (Figure 2c), and the group in the *meso* position does not affect the absorption properties.

Fluorescence quantum yield (Φ_F) of **1** – **3** were determined in different solvents, and compounds showed similar Φ_F values (Table 1). The decays of the fluorescence fit to a single exponential function, revealing single excited state lifetime in the range 2.5 – 3.8 ns.

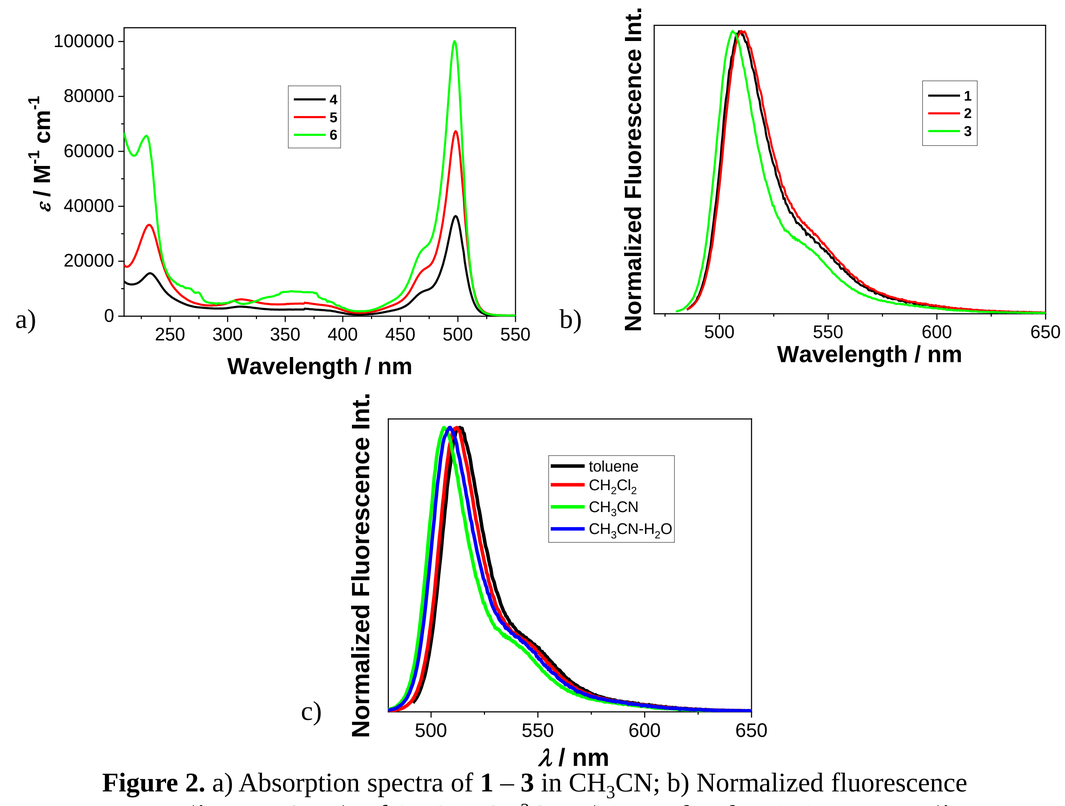


Figure 2. a) Absorption spectra of **1** – **3** in CH_3CN; b) Normalized fluorescence spectra (λ_{exc} = 470 nm) of **1** – **3** in CH^3CN; c) Normalized emission spectra (λ_{exc} = 470 nm) of **3** in four different solvents.

Table 1. Photophysical properties of BODIPY dyes **1** – **3**.

Compound	Solvent	Φ_F [a]	τ/ns [b]
1	Toluene	0.80 ± 0.05	2.78 ± 0.01
	CH_2Cl_2	0.70 ± 0.02	2.73 ± 0.02
	CH_3CN	0.55 ± 0.01	2.60 ± 0.02
	CH_3CN-H_2O (1:10)	0.51 ± 0.05	3.00 ± 0.01
2	Toluene	0.84 ± 0.06	2.81 ± 0.01
	CH_2Cl_2	0.75 ± 0.02	2.59 ± 0.02
	CH_3CN	0.50 ± 0.01	2.57 ± 0.02
	CH_3CN-H_2O (1:10)	0.51 ± 0.02	2.70 ± 0.01
3	Toluene	0.91 ± 0.06	3.46 ± 0.01
	CH_2Cl_2	0.78 ± 0.02	3.45 ± 0.01
	CH_3CN	0.54 ± 0.06	3.37 ± 0.01
	CH_3CN-H_2O (1:10)	0.62 ± 0.03	3.83 ± 0.01

[a]Quantum yield of the fluorescence measured by use of Rhodamine B in methanol as a reference (Φ_F = 0.66), [b]Lifetime of the singlet excited state measured by TC-SPC.

CONCLUSION

- simple peptide coupling protocol for the synthesis of BODIPY amide derivatives **1** and **2**
- click chemistry for the synthesis of BODIPY derivative **3**
- excellent spectral and photophysical properties for all three compounds
- selective localization into intracellular organelles (lysosome, endoplasmic reticulum, and mitochondria)

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