

Exploring BODIPY *meso*-enamines as singlet-oxygen photosensitizers for PDT.

Eduardo Palao,¹ Ruth Prieto-Montero², Alejandro Prieto-Castañeda¹, Nerea Epelde-Elezcano², Antonia R. Agarrabeitia¹, Florencio Moreno¹, Beatriz L. Maroto¹, Virginia Martínez-Martínez^{2,*}, Santiago de la Moya^{1,*} and María J. Ortiz^{1,*}

¹ Departamento de Química Orgánica I, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Ciudad Universitaria s/n, 28040, Madrid, Spain

² Departamento de Química Física, Universidad del País Vasco-EHU, Apartado 644, 48080, Bilbao, Spain.

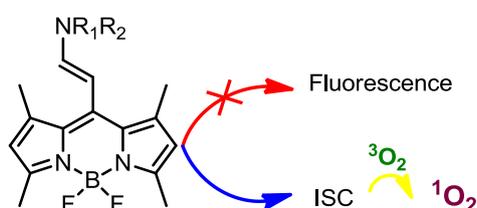
* Correspondence: mjortiz@ucm.es, santmoya@ucm.es; virginia.martinez@ehu.es Tel.: +34-913944309, +34-913945090.

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Abstract: The recent access to the control of Vilsmeier-Haack reaction in BODIPYs has opened the selective generation of *meso*-enamines BODIPYs. *meso*-enamine functionalization deeply alters the photophysical properties of the BODIPY by quenching efficiently the fluorescence by the activation of non-radiative de-excitation channels, which in turn enables singlet-oxygen generation, and makes *meso*-enamine BODIPYs interesting as a possible new family of structurally-simple, easily-accessible and halogen-free singlet-oxygen photosensitizers for PDT. In this communication, we present the first study on the capability of a series of *meso*-enamines BODIPYs to generate singlet oxygen.



Keywords: BODIPYs, Singlet oxygen photosensitizers, PDT

1. Introduction

Production of singlet oxygen (¹O₂) through photosensitization involves three components: a photosensitizer (PS), a light source and oxygen. The development of ¹O₂ photosensitizing agents is an active research field since ¹O₂ is an useful reactive specie for numerous applications such as oxidation of fine chemicals in organic solvents [1], wastewater treatment [2] or cancer treatments by photodynamic therapy (PDT) [3]. Boron dipyrromethene (BODIPY) dyes have emerged as relevant fluorophores and ¹O₂ photosensitizers [3b,4]. Their suitability for such applications are due to their very favorable chemical and physical features, including high extinction coefficients in the visible region, resistance to photobleaching [5], resilience to environmental conditions (solvent polarity or pH) and high lipophilicity [6]. Moreover, their synthetic versatility allows exhaustive and selective functionalization of the chromophore in order to modulate their properties. For instance, chemical

modifications can be conducted to increase $^1\text{O}_2$ generation to achieve the therapeutic efficiency required for PDT [3a,3b].

The generation of reactive oxygen species as $^1\text{O}_2$ requires efficient population of the dye excited triplet-state. In general, BODIPYs do not undergo efficient intersystem crossing (ISC) to such an excited state, which can be accomplished by involving heavy atoms in the dye structure [3b,5,7,8](Figure 1). Unfortunately, the presence of heavy atoms could promote dark toxicity, which makes difficult the implementation of the BODIPY dye as a clinic photosensitizer for PDT [9]. As an alternative, Flamigni and coworkers synthesized the first halogen-free BODIPY photosensitizers, which are based on orthogonal-BODIPY dimer [10]. Their unique properties have promoted a growing interest in developing new halogen-free BODIPYs for PDT [11]. In this line, our research group has deeply studied the ability of orthogonal-BODIPY dimers to generate $^1\text{O}_2$, and has postulated that it occurs through a spin-orbit charge-transfer intersystem crossing (SOCT-ISC) mechanism [12]. In this sense, singlet oxygen generation is demonstrated to be strongly connected with the intrinsic ICT character of the dye and, consequently, it can be used to design new halogen-free PDT agents based on BODIPY (Figure 1).

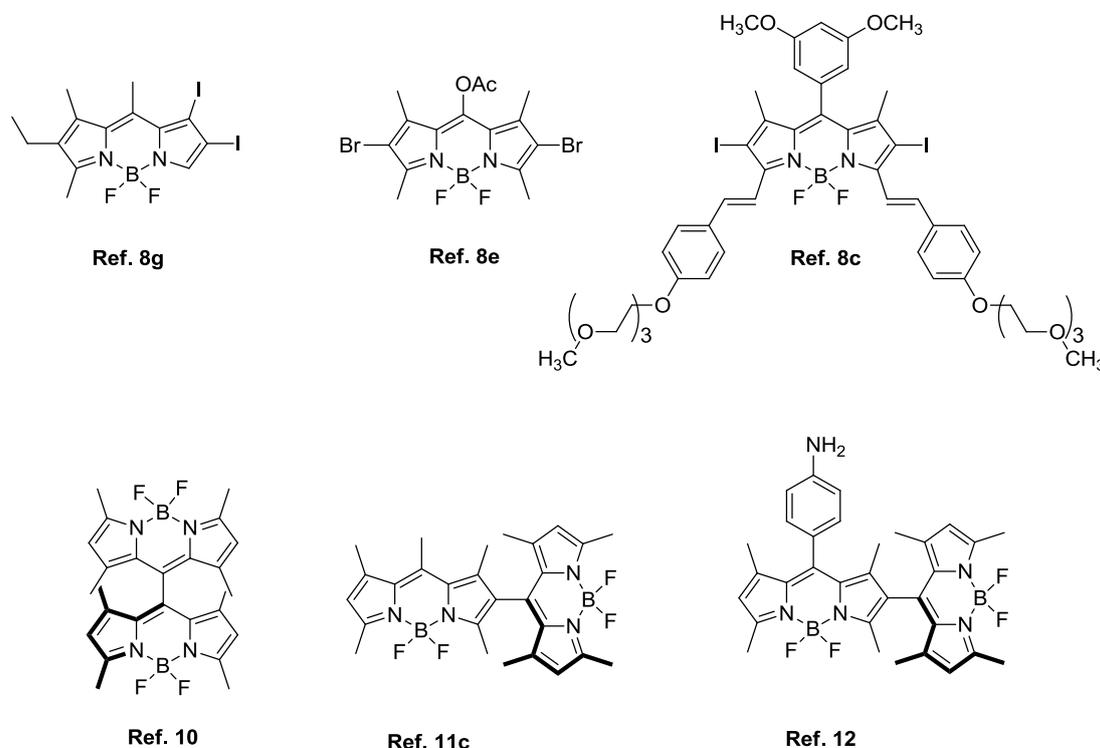


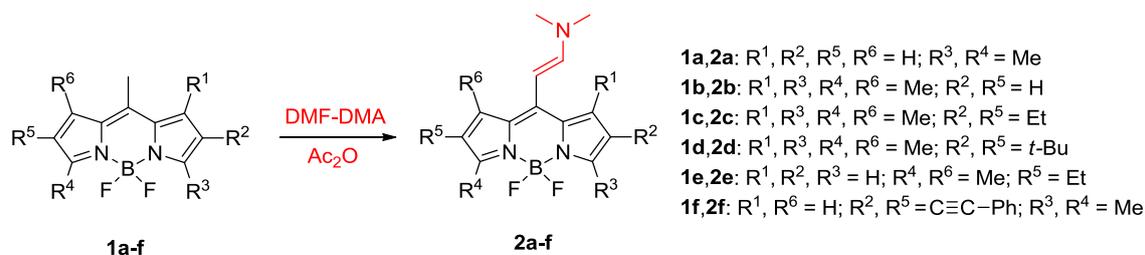
Figure 1. Some examples of haloBODIPYs and BODIPY dimers described.

On the other hand, we have recently reported on the easy access to BODIPY *meso*-enamines from *meso*-methylBODIPYs by selective Vilsmeier-Haack reaction [13]. The obtained enamines were non-fluorescent, which was attributed to the switching-on of non-radiative deactivation channels, like ICT, which in turn could make possible the generation of $^1\text{O}_2$ without involving heavy atoms or complex polyBODIPY designs, as it was preliminary demonstrated [13]. This interesting result prompted us to explore the capability of further BODIPY *meso*-enamines to generate $^1\text{O}_2$, by synthesizing and studying a selected series of these compounds (see Scheme 1). This work shows the most interesting results concerning this study.

2. Results

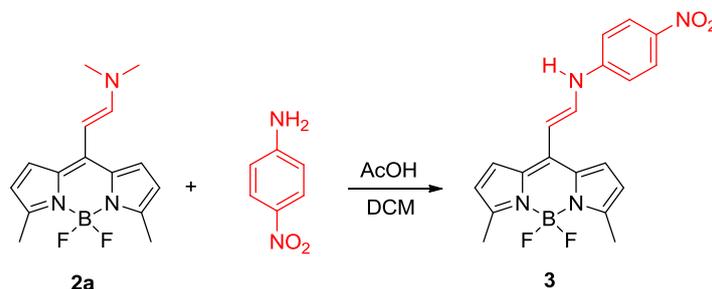
2.1. Synthesis of *meso*-enamines

BODIPYs **1a** [14], **1c** [15] and **1f** [13] were synthesized by the methods previously described, BODIPYs PM546 (**1b**), PM567 (**1d**) and PM597 (**1e**) are commercially available (laser grade; purity > 99%), and were used as received. From them, using DMF dimethyl-acetal in acetic anhydride (DMF-DMA/Ac₂O), as selective electrophilic reagent [13], allows the selectively attainment of the serie of *meso*-enamine BODIPY shown in Scheme 1 (**2a-f**).



Scheme 1. Synthesis of *meso*-enamine BODIPYs **2a-f** from *meso*-methylBODIPYs **1a-f**.

These transamination reactivity of dimethylamino-based enanamines, [16] allowed extending the study series by the preparation of enamine **3**, which was straightforwardly obtained by treating **2a** with 4-nitroaniline in presence of acetic acid and dichloromethane (DCM) as the solvent (Scheme 2).



Scheme 2. Synthesis of *meso*-enamine BODIPY **3** from *meso*-enamine BODIPY **2a**

2.2. Photophysical properties and singlet oxygen production

The obtained enamines were photophysically and photochemically (singlet oxygen generation) characterized in DCM diluted solution. The obtained data are summarized in Table 1. 5-Dimethyl-2,6-diiodo-8-thiomethyl-pyrromethene in the same solvent was used as the reference for ¹O₂ quantum yield (Φ_Δ = 0.87) [8d].

Symmetric alkylation of the BODIPY core induces important change in the absorption spectra of the compounds (Figure 2). In this sense, a new shoulder at higher energy with respect to the main absorption band is observed, which is assigned to the contribution of an iminium-like form (see Figure 2 inset). In other words, in these compounds the recorded two absorption bands are the result of the participation of two main forms (iminium-like and enamine-like) [13]. The presence of the iminium-like form increases with the alkylation degree of the chromophore, as it is confirmed by a more intense high-energy shoulder in the absorption spectrum, following the order 2a < 2b < 2c < 2d

(see Figure 2 and Table 1). However, the higher the contribution of the iminium-like form, the lower the $^1\text{O}_2$ production (Table 1).

Table 1. Photophysical and photochemical ($^1\text{O}_2$ production) properties of BODIDY *meso*-enamines in diluted (ca. 10^{-6}M) DCM solution; absorption wavelength (λ_{ab}), molar absorption (ϵ_{max}), fluorescence wavelength (λ_{fl}), fluorescence quantum yield (Φ_{flu}) and $^1\text{O}_2$ quantum yield (Φ_{Δ}).

BODIPY	λ_{ab} (nm)	ϵ_{max} ($10^4 \text{ M}^{-1} \text{ cm}^{-1}$)	λ_{fl} (nm)	Φ_{flu}	Φ_{Δ}
2a	492.0	6.6	515.5	<0.01	0.12
2b	487.0	4.8	515.0	<0.01	0.07
2c	453.0 (47%)	3.3	543.0	<0.01	0
	510.0 (53%)	3.9			
2d	458.0 (53%)	0.6	549.0	<0.01	0
	517.0 (47%)	0.6			
2e	498.0	0.7	522.0	<0.01	0.12
2f	540.0	3.0	599.0	0.12	0.18
3	507.0	2.8	512.0	0.05	0.17

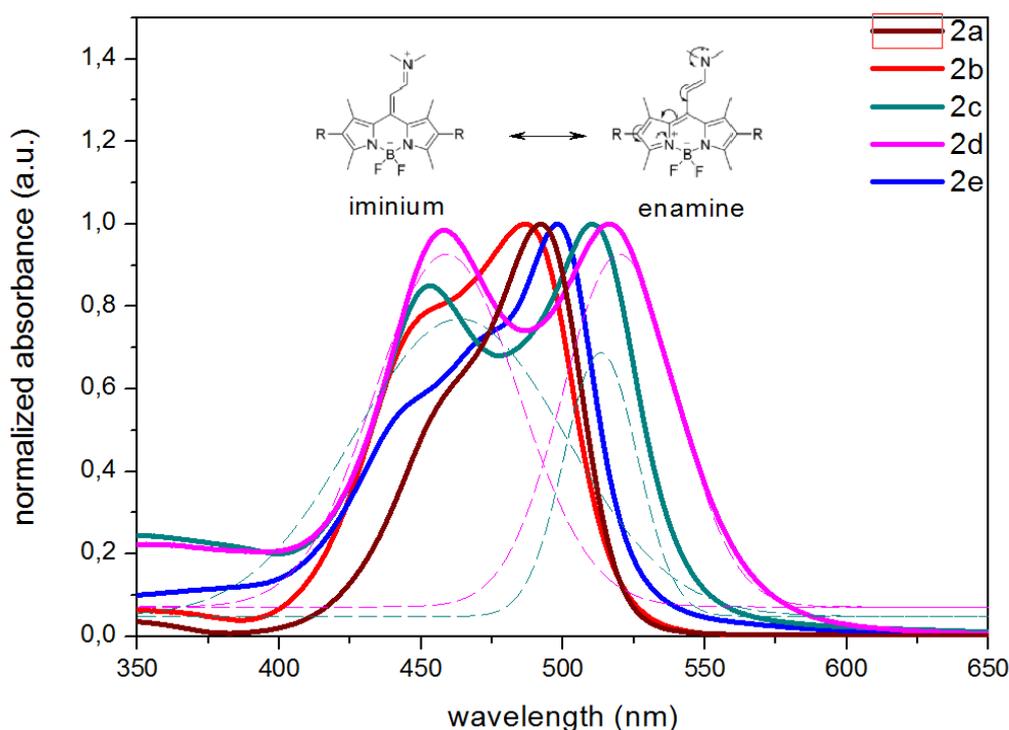


Figure 2. Absorption spectra of BODIPYs 2a, 2b, 2c, 2d and 2e in DCM. Inset iminium- and enamine-like structures.

This fact is attributed to the decrease of the negative charge located at the dimethylated nitrogen, reducing the inherent push-pull nature of the chromophore, therefore decreasing the ICT process responsible of the $^1\text{O}_2$ generation. Strikingly, the asymmetric alkylation of the BODIPY core (2e) does not favored the said iminium-like form in the same way, and the negative charge located at

the dimethylated nitrogen is not practically modified as supported by computational calculation (data not shown), and the $^1\text{O}_2$ production is recovered (similar to that measured for **2a**).

Additionally, introducing of phenylethynyl groups at positions 2 and 6 of BODIPY **2a**, to generate **2f**, not only extends the chromophoric π -conjugation, causing a significant bathochromic shift ($\lambda_{\text{ab}} = 540 \text{ nm}$ in **2f** vs. $\lambda_{\text{ab}} = 492 \text{ nm}$ in **2a**; cf. spectrum in Figures 2 and 3), but, more interestingly, increases also the production of $^1\text{O}_2$ (Φ_{Δ} up to ca. 20%; see Table 1) at the same time that a moderate fluorescence efficiency is maintained ($\Phi_{\text{fl}} = 0.15$; see Table 1). This dual functionality is useful for theragnostic applications based on diagnosis by fluorescence imaging and therapy by PDT. In this line, it must be noted that both dual properties can be modulated in BODIPY *meso*-enamines by selecting properly the BODIPY substitution pattern.

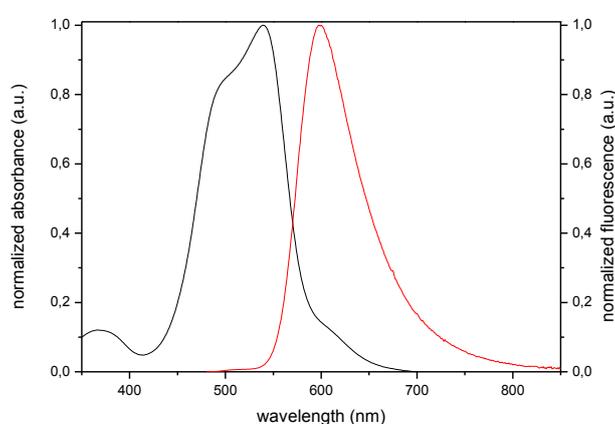


Figure 3. Absorption spectra and emission spectra of **2f** in dichloromethane

The contribution of the iminium-like form can be also diminished by introducing electron-withdrawing groups connected to the amino rest, as it occurs in **3** involving a 4-nitroaniline rest (cf. $\Phi_{\text{fl}} = 0.05$ and $\Phi_{\Delta} = 0.17$ for **3** vs. $\Phi_{\text{fl}} < 0.01$ and $\Phi_{\Delta} = 0.12$ for **2a**).

3. Conclusions

meso-Enamine BODIPYs are a new family of structurally-simple and easily-accessible BODIPYs with capability to act as halogen-free $^1\text{O}_2$ photosensitizers for PDT or even for theragnosis based on PDT + fluorescence. On the other hand, both dual opposite properties, $^1\text{O}_2$ production and fluorescence efficiency can be finely modulated by easy chemical modification affecting the inherent ICT character of the involved BODIPY chromophore. Further investigations are conducted to develop efficient BODIPY *meso*-enamines for clinic PDT and theragnosis.

Supplementary Materials: Supplementary Methods, Supplementary Figure and Supplementary References.

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Authors Contributions: M. J. O., S. M. C. conceived the new material, designed its synthesis, and supervised the overall work. E. P., A. P.-C., S. M., F. M. and B. L. carried out the experimental work related with the material preparation and chemical characterization. V. M.-M. designed and supervised the work concerning the photophysical characterization of the material. N. E.-E. and R. P.-M. conducted experimental work regarding $^1\text{O}_2$ -generation activity. All authors contributed to the preparation of the manuscript. M. J. O., S. M. C. and V. M.-M. made major edits on the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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