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Nitrosation of BODIPY dyes and their applications in the development of thiol sensors

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A B S T R A C T

Herein we describe a new method for the post-functionalization of BODIPY dyes, by means of nitrosation with $NOBF_4$, in yields of up to 93%. The application of nitroso-BODIPY dyes as analytical tools for the fluorimetric analysis of thiols is also shown, including the ability to cross cell membranes.

1. Introduction

BODIPYs play an important role in biological sciences and chemical biology as chemosensors and biological markers [1,2]. While the dipyrromethene core of BODIPYs is very reactive allowing introduction of different functional groups, nitroso substitution of BODIPYs is unprecedented in the literature, and direct nitrosation opens opportunity for the development of new fluorescent probes. Although several methods for nitrosation of aromatic compounds are available [3], the nitroso functionalization of electron-rich aromatic rings (π-excessive), as pyrroles, is a synthetic challenge, with only few described examples in the literature [4].

Nitroso-containing coumarin were recently reported as useful probes for H_2S, due to sulfide-mediated reduction of the nitroso group [5]. However, fluorescence-based analytical methods involving nitroso dyes are not fully described for thiol containing organic compounds, such as cysteine (Cys) and glutathione (GSH) and would be an important tool for analysis of wide range of cellular processes [6,7].

Based on the recognized reactivity of BODIPYs with electrophiles, in this paper we describe a fast and efficient method for the nitrosation of the pyrrole ring in BODIPYs core using $NOBF_4$ [8]. In addition, we studied the reactivity and photophysical properties of nitroso-BODIPY in the presence of biothiols.

2. Results and discussion

2.1. Study of the nitrosation of BODIPY

Nitrosation reactions were carried out with different substituted BODIPYs, which were synthesized using previously published methods (Table 1).

Initially, we studied the direct nitrosation of 1,3,5,7,8-pentamethyl BODIPY **1** using $NOBF_4$ as a nitroso source, in different conditions (solvents, temperature and atmosphere), as described in Table 2. Acetonitrile was the most suitable solvent, yielding 65% of the 2-nitroso BODIPY **2**, with full conversion of **1** in 10 min at −5 °C (Entry 6), which is possibly related to the higher solubility of $NOBF_4$ in this solvent. Room temperature and open-vessel conditions were detrimental (Entries 7 and 8), while no relevant change in yields was observed when the reaction was carried out at −40 °C (Entry 9).

Another classical method for nitrosation of aromatic rings, using sodium nitrite and hydrochloric acid ($NaNO_2$/HCl) was evaluated. However, nitroso compounds were not observed in any of the reactions, but the reactions resulted in degradation of the BODIPYs starting materials.

Based on the successful nitrosation of **1**, we tested the optimized method for the modification of the symmetric BODIPYs **3–6**. These experiments showed that nitrosation of 1,3,5,7-tetramethyl-8-aromatic BODIPYs with $NOBF_4$ is a straightforward reaction regardless of the aromatic substituent, with moderate to high yields (Scheme 1).

The nitrosation was successfully applied to symmetric non-methylated BODIPY **12**, from which two regioisomers were produced (Scheme

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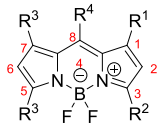
0143-7208/

Table 1
Structure numbering of the BODIPY core and starting materials used in the nitrosation study.



#	R^1	R^2	R^3	R^4	Ref.
1	Me	Me	Me	Me	[9]
3	Me	Me	Me	4-fluorophenyl	[10]
4	Me	Me	Me	4-cyanophenyl	[10]
5	Me	Me	Me	4-methoxyphenyl	[10]
6	Me	Me	Me	Ph	[10]
12	H	H	H	Ph	[11]
13	H	H	H	2,6-dichlorophenyl	[11]
20	H	Ph	H	Ph	[12]

2). Compound **14**, substituted at C-3, was isolated with 40% yield, while the 2-nitroso BODIPY **16** was a minor regioisomer, isolated with 19% yield. On the other hand, for the nitrosation of the structurally-related compound **13**, the reaction was regioselective, furnishing **15** in higher yields (78%). It is interesting to note that electrophilic substitutions, such as nitration, sulfonation, formylation and thiocyanation, are usually observed at C-2 and C-6 of the BODIPY core [2a,13-17], while at C-3 and C-5 are prone to nucleophilic substitutions [18]. However, there is one report showing nitration at C-3, using HNO_3 in Ac_2O at low temperature, supporting the possibility of electrophilic substitutions at this position [19]. In general, moderate to excellent yields were obtained for that conversion compared to other electrophilic substitution reactions described for BODIPYs [19].

The regioisomers **14** and **16** were easily differentiated by ^{1}H NMR spectroscopy, especially when analyzing the signals of hydrogens at C-2, C-3, C-5 and C-6. The structure of **14** was confirmed by ^{1}H NMR spectra (400 MHz, $DMSOd_6$ - in the supporting information – SI page 15), showing chemical shifts observed for hydrogen C-5 at 8.80 ppm (s, 1H); C-2 at 7.34 ppm (dd, J = 4.5 Hz, 1H); C-6 at 7.06 ppm (dd, J = 4.7, 1.6 Hz, 1H). For compound **16** the ^{1}H NMR spectra (400 MHz, $DMSOd_6$ - in the supporting information - SI page 17), show the chemical shifts observed for hydrogen at C-5 at 8.74 ppm (s, 1H); C-3 at 8.74 ppm (s, 1H); C-6 at 7.01 ppm (d, J = 4.6 Hz, 1H). Analyzing these results, for **14** there is no signal around 8 ppm, referent to hydrogen at C-3 on the starting material. On the other hand, for **16**, no signal around 7 ppm with ^3J referring to typical coupling constant ββ' typical in pyrrole, suggesting the regioisomers (Scheme 2).

Aiming to expand the scope of this nitrosation method, we carried out the reaction with the asymmetric BODIPY **20**. Interestingly, this reaction followed the same reactivity shown above, and furnished regioselectivity asymmetric red-shifted 3,8-diphenyl BODIPY **20**, which yielded 73% of the nitrosated BODIPY **21**. The formation of 2-nitroso-substituted derivative, which was expected based on the previous experiments in this work, was not observed (Scheme 3).

According to the described reactivity of BODIPYs in the literature [16,20], the nitrosation reaction is probably based on an electrophilic attack at the nitrosonium cation, while the observed regioselectivity can be explained by the favourable formation of the intermediates **25a – 25c** (Scheme 4) compared to **24a – 24b**. Furthermore, the successful nitrosation with $NOBF_4$ may reflect the importance of tetrafluorborate as a counteranion in this reaction. In this context, a six-membered cyclic transition state could be involved during the reaction process (Scheme 4).

According to the photophysical experiments, nitroso-BODIPYs invariably showed reduced fluorescence quantum yields, compared to the parent starting materials, possibly due to the addition of a new non-radiative decay pathway. As expected, fluorescence quantum yield was even lower for non-methylated BODIPYs, due to the free rotation of the meso-substituent that leads to non-radiative decay of the excited state [21]. It is worth of note that nitrosation was not related to relevant shifts in the emission and absorption spectra (in the supporting information – SI pages 4–8).

2.2. Reactivity of nitroso-BODIPY and fluorescence properties involving compounds with thiol

Considering that nitroso group is reactive towards thiols, leading to the reduced product [5], the reactivity and photophysics of the products synthesized herein were studied. As a model, compound **21** was reacted with sodium hydrosulfide (NaHS) in different concentrations, and a relevant increase in the emission (detailed in the SI pages 2–3) was observed. Through this experiment, the concentration of thiol analytes was established for use in further experiments.

Therefore, we analyzed the fluorescence of a solution of **15** (0.5 mM) in MeOH/Water 1:1 in the presence of thiopropanol (50 mM). Interestingly, we observed an increased and red-shifted emission outcome from the interaction of **15** with thiopropanol. Similar changes in the emission were observed for solutions of **15** treated with cysteine (Cys), *N*-acetylcysteine (NAC), glutathione (GSH) and 2-mercaptopyridine, while no relevant changes were observed for solutions treated with ethylamine or other amino acids (Fig. 1). Interestingly, no changes in fluorescence were observed for the cystine (oxidized dimer of cysteine), showing the importance of the free thiol for nitroso BODIPYs interactions. These observations reinforce the idea that **15** can act as a specific fluorescent probe for biothiols.

From visual inspection (SI - page 9), it is clear that the emission of **15** is particularly enhanced by NAC and GSH, while the effect for Cys is smaller (Fig. 2). Interestingly, the fluorescence emission of other nitrosyl BODIPYs (**11**, **21**, **14**) were also shown to be relevantly changed by the treatment with these three biothiols (Fig. 2). On the other hand, we observed that 2-substituted dye **16** showed no change in fluorescence, under studied conditions, a reason for this behaviour may be related to compound decomposition, which is coherent to the literature data [19].

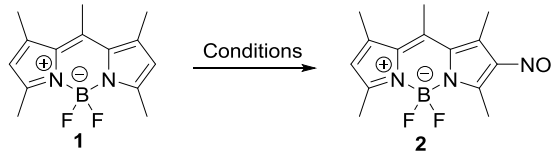
To understand the response of nitroso-substituted BODIPYs when in the presence of thiol compounds, the reaction between **15** and thiopropanol was further investigated. Remarkably, TLC control showed the formation of a mixture of fluorescent red-shifted products. From this mixture, it was possible to isolate and confirm the structure of 3-amino,5-thiopropyl **26** (Scheme 5). Regarding the obtained compound **26**, it can be suggested that the process involving nitroso-BODIPY and thiol group can be related to two processes that may occur concomitantly, one of them being the addition of the thiol group to the pyrrole ring of BODIPY, with further reduction of the NO group, as observed for compound **26**.

The NMR ^{1}H analyses (in the supporting information – SI pages 20–21) suggests that three other compounds were formed, having signals related to the structure of the BODIPY core. It is interesting to note, the addition of thiopropanol to C-5 of the BODIPY, which has recently been reported when using an oxidizing agent [22].

To identify if compound **26** was responsible for the increase in fluorescence in thiol sensor experiments, the emission spectra of the pure product **26**, **15** and the spectrum of the thiol-sensor experiment solution (Mixture of **15** with thiopropanol – 589 nm) were compared (Fig. 3).

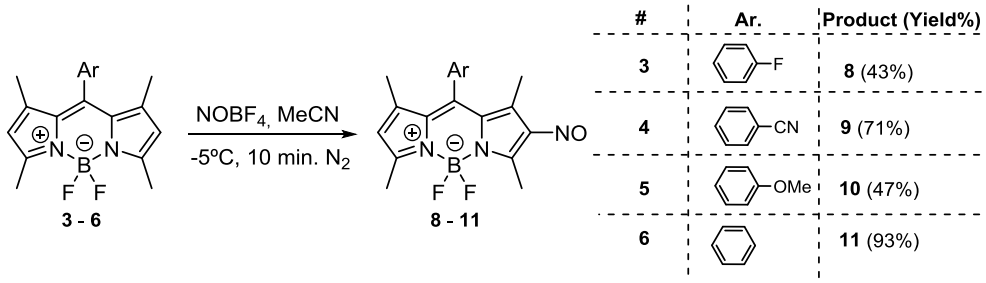
Pure compound **26** showed fluorescence emission (569 nm) which is far from the observed in the thiol-sensor experiment (589 nm), however, when compared with **15** (534 nm), it resulted in an expected bathochromic shift due to the insertion of the thiol group. In the literature, It is well-described that 3-thio-substituted BODIPYs are commonly associated with relatively intense and red-shifted fluorescence emission [23b]. On the other hand, the same is not reported for 3-amino-substituted BODIPYs [23], which usually show less intense

Table 2
Optimization of the nitrosation of BODIPY **1**.

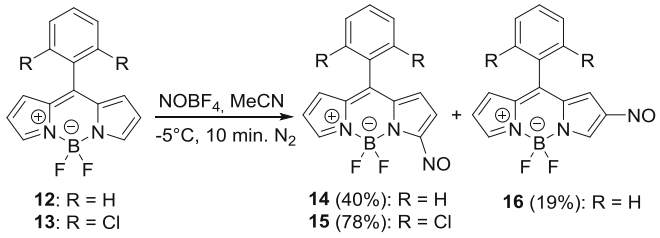


Entry	Conditions[a]				Yield[b]
	Solvent	Temp.[c]	Atm.	Time[d]	
1	DCE	-5[e]	N_2	45	25
2	1,4 -Dioxane	-5[e]	N_2	35	27
3	DMF	-5[e]	N_2	120	0
4	Me_2CO	-5[e]	N_2	15	31
5	THF	-5[e]	N_2	40	30
6	MeCN	-5[e]	N_2	10	65
7	MeCN	rt	N_2	10	36
8	MeCN	-5[e]	Air	10	47
9	MeCN	−40[f]	N_2	15	68

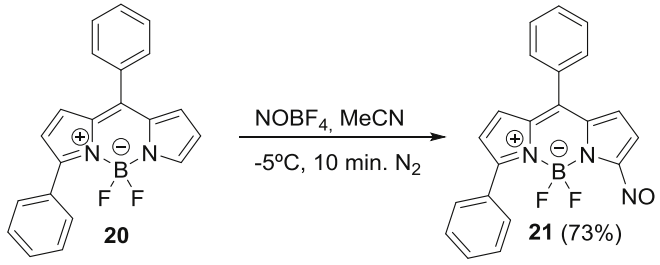
[a] Reactions were carried out with 50 mg of compound **1** and 1.5 eq. of $NOBF_4$.
[b] Calculated yield % after chromatographic purification.
[c] Temperatures are given in °C or rt (room temperature).
[d] Time in minutes for complete consumption of starting material according to TLC control.
[e] Ice bath/acetone.
[f] Dry ice bath/acetonitrile.



Scheme 1. Nitrosation of 1,3,5,7-tetramethyl-8-aromatic BODIPYs.



Scheme 2. Nitrosation of non-methylated BODIPYs.

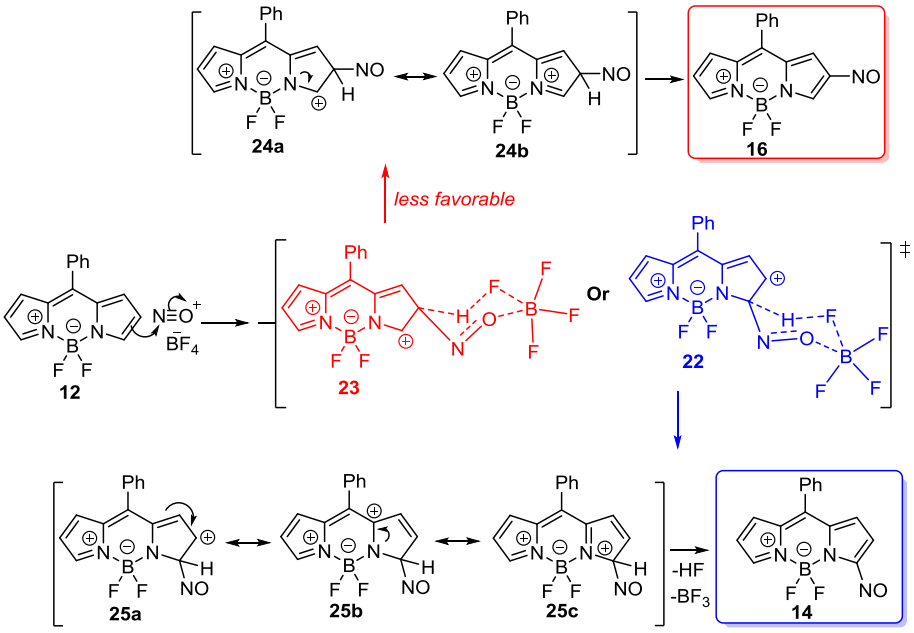


Scheme 3. Nitrosation of 3,8-diphenyl BODIPY.

fluorescence because the electron releasing capacity of the amino at C-3 is not strong enough to induce a charge transfer state, which usually drastically quenches the fluorescence emission of BODIPYs [19]. Nitroso-BODIPYs produced herein invariably showed reduced fluorescence quantum yields, compared to the parent starting materials.

2.3. Cellular uptake of nitroso-BODIPY

In addition, we analyzed the ability of the nitroso-BODIPY to cross cell membrane, to access their usefulness as *in vitro* probes. In that sense, a flow cytometry analysis was performed in A431 squamous cell carcinoma line, as a model tumour cell line, as shown in Fig. 4A–C flow cytometry dot plots. Thiol-probe **15** was used as treatment for a duration of 5 min, 30 min and 24 h. A time-dependent permeation process was observed (Fig. 4), with the highest amount of **15** positive cells at 24 h (Fig. 4D). This result was also confirmed by the fluorescence intensity at λ_{em} = 585/42 nm (Fig. 4E) at the end of the experiment, which is similar to the observed for the solution thiol-sensor experiment (589 nm). Statistical difference was observed for the different treatment durations ($p < 0.05$, One-way ANOVA with Tukey's multiple comparison test) and no viability cell changes were observed. As a result, the compound **15** presents time-dependent tumoral cellular uptake and was effective in acting as an *in vitro* probe.



Scheme 4. Mechanistic proposal for BODIPY nitrosation.

3. Concluding remarks

This work showed the development of a simple and very efficient method for obtaining the unprecedented nitroso-substituted fluorophores, with up to 93% yields, using a readily available and inexpensive reagent. In addition, a preliminary chemical reactivity study involving thiols yielded the reduced nitroso to amino group, with an unexpected addition to the BODIPY core. These structural changes and unusual reactivity for BODIPYs modulate the changes in photophysical properties, which could be also followed by cell studies, indicating the ability of the nitroso BODIPY to cross cell membranes, instigating a future study of the potential sensor for thiol groups. Finally, these results demonstrate the potential application of nitroso-BODIPY dyes as fluorescent probes and encourages us to continue studying this class of compounds for further applications.

4. Experimental section

4.1. General procedure 1: optimized nitrosation reaction

$NOBF_4$ (1.5 eq.) was added to a stirring solution of the BODIPY in

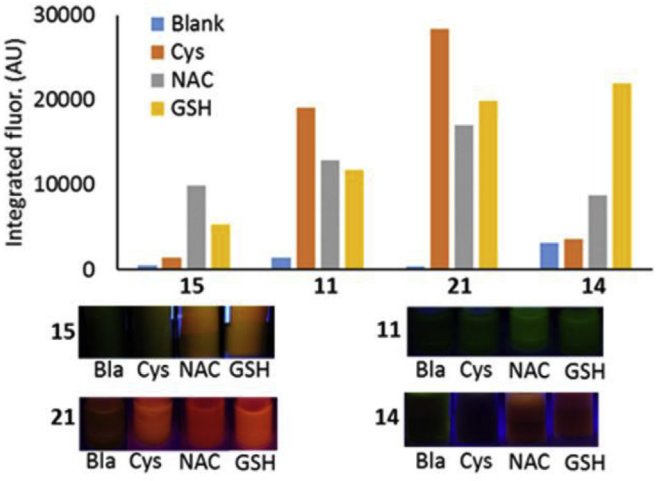


Fig. 2. Changes in the fluorescence emission intensity of compounds **15**, **11**, **21** and **14** after treatment with thiols. Bla: Blank. Conditions: MeOH/H_2O 1:1, concentrations: 0.5 mM of nitroso-BODIPYs and 50 mM of biothiols.

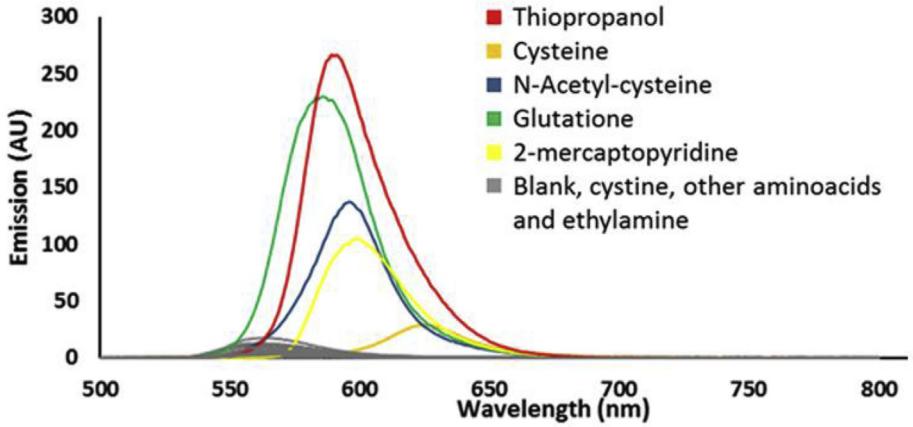
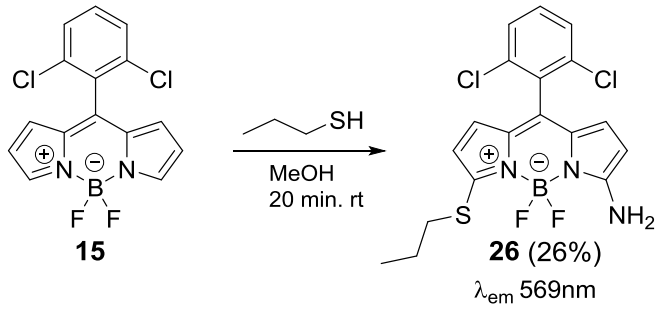


Fig. 1. Changes in the fluorescence emission of compound **15** after treatment with thiols. Conditions: MeOH/H_2O 1:1, concentrations: 0.5 mM of nitroso-BODIPYs and 50 mM of biothiols.



Scheme 5. Reaction of BODIPY **15** with Thiopropanol.

dry acetonitrile (5 mL) cooled to -5 °C (Ice bath/acetone), under nitrogen atmosphere, and the reaction course was followed by TLC. After full consumption of the starting material, water was added, and the solution was left stirring for 5 min. The reaction mixture was extracted three times, with EtOAc, dried over magnesium sulfate and concentrated to dryness. The residue was purified chromatographically in a silica gel column, using mixtures of Hex:EtOAc or Hex:DCM as eluents, to yield the desired product.

*4.1.1. 1,3,5,7,8-Pentamethyl-2-nitrosyl-BODIPY **2***

Prepared from BODIPY **1** (50 mg, 0.190 mmol) using the optimized nitrosation reaction in 65% yield (36 mg, 0.123 mmol); m.p 240–249 °C. (C_6H_{14}/EtOAc 8:2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.30 (s, 1H), 2.80 (s, 3H), 2.71 (s, 3H), 2.70 (s, 3H), 2.59 (s, 3H), 2.49 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 162.6, 147.8, 147.0, 143.8, 139.0, 136.0, 132.2, 128.3, 125.3, 18.2, 17.8, 15.2, 14.5, 14.3. IR (neat): 1568, 1530, 1479, 1411, 1376, 1337, 1196, 1150, 1066, 980 cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{14}H_{16}BF_2N_3O$ $[M + H]^+$ 292.1427, found 292.1428.

*4.1.2. 1,3,5,7-Tetramethyl-2-nitrosyl-8-(4-fluorophenyl)-BODIPY **8***

Prepared from BODIPY **3** (50 mg, 0.146 mmol) using the optimized nitrosation reaction in 43% yield (24.3 mg 0.063 mmol); m.p 130–140 °C. (C_6H_{14}/EtOAc 8:2). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.54 (dd, J = 8.0, 5.6 Hz, 2H), 7.46 (t, J = 8.7 Hz, 2H), 6.57 (s, 1H), 2.73 (s, 3H), 2.59 (s, 3H), 1.60 (s, 3H), 1.44 (s, 3H). ^{13}C NMR (101 MHz, $DMSOd_6$) δ 165.9, 164.6, 162.1, 149.4, 147.8, 142.9, 138.7, 135.4, 133.5, 130.9, 130.8, 129.6, 129.6, 126.9, 126.2, 117.3, 117.1, 15.4, 15.2, 14.3, 12.3. IR (neat): 2955, 2925, 2856, 1734, 1603, 1521, 1334, 1310, 1260, 1190, 1068, 813 cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{19}H_{17}BF_3N_3O$ $[M + H]^+$ 372.1490, found 372.1494.

*4.1.3. 1,3,5,7-Tetramethyl-2-nitrosyl-8-(4-cyanophenyl)-BODIPY **9***

Prepared from BODIPY **4** (43 mg, 0.113 mmol) using the optimized nitrosation reaction in 71% yield (28 mg, 0.080 mmol); m.p 258–262 °C. (C_6H_{14}/EtOAc 8:2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.89 (d, J = 8.3 Hz, 2H), 7.51 (d, J = 8.2 Hz, 2H), 6.29 (s, 1H), 2.95 (s, 3H), 2.67 (s, 3H), 1.69 (s, 3H), 1.44 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 166.1, 165.0, 147.9, 147.9, 142.0, 138.6, 136.8, 135.3, 133.4, 129.2, 127.6, 126.0, 117.8, 114.3, 15.6, 15.4, 13.0, 11.3. IR (neat): 2970, 2955, 2832, 1554, 1310, 1191, 1185, 1069, 989, 815 cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{20}H_{17}BF_2N_4O$ $[M + H]^+$ 379.1536, found 379.1538.

*4.1.4. 1,3,5,7-Tetramethyl-2-nitrosyl-8-(4-methoxiphenyl)-BODIPY **10***

Prepared from BODIPY **5** (100 mg, 0.282 mmol) using the optimized nitrosation reaction in 47% yield (51 mg, 0.133 mmol); m.p 225–230 °C. (C_6H_{14}/EtOAc 8:2). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.19–7.13 (m, 2H), 7.09–7.02 (m, 2H), 6.20 (s, 1H), 3.89 (s, 3H), 2.85 (s, 3H), 2.63 (s, 3H), 1.71 (s, 3H), 1.49 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.1, 160.8, 149.1, 148.9, 144.0, 139.0, 135.5, 134.9, 129.0, 127.5, 125.62, 124.8, 115.0, 55.4, 15.3, 14.3, 14.3, 12.6. IR (neat): 2960, 2925, 2843, 1609, 1564, 1536, 1487, 1417, 1336, 1245, 1171, 1087, 1017, 841 cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{20}H_{20}BF_2N_3O_2$ $[M + H]^+$ 384.1689, found 384.1678.

*4.1.5. 1,3,5,7-Tetramethyl-2-nitrosyl-8-phenyl-BODIPY **11***

Prepared from BODIPY **6** (50 mg, 0.154 mmol) using the optimized nitrosation reaction in 93% yield (51 mg, 0.144 mmol); m.p 190–195 °C. (C_6H_{14}/DCM 7:3). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.59–7.50 (m, 3H), 7.33–7.27 (m, 2H), 6.25 (s, 1H), 2.97 (s, 3H), 2.65 (s, 3H), 1.69 (s, 3H), 1.46 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.4, 164.9, 148.9, 148.8, 145.0, 135.8, 133.7, 129.9, 129.7, 128.4, 127.7, 125.3, 111.8, 15.5, 15.2, 13.0, 11.0. IR (neat): 2961, 2926, 2851, 1556, 1415, 1335, 1310, 1261, 1190, 1144, 1064, 996, 824, 722 cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{19}H_{18}BF_2N_3O$ $[M + Na]^+$ 376.1398, found: 376.1402.

*4.1.6. 3-Nitrosyl-8-phenyl-BODIPY **14***

Prepared from BODIPY **12** (70 mg, 0.261 mmol) using the optimized nitrosation reaction in 40% yield (31 mg, 0.104 mmol); m.p 175–182 °C. (C_6H_{14}/EtOAc 7:3). ^{1}H NMR (400 MHz, $DMSOd_6$): δ 8.80

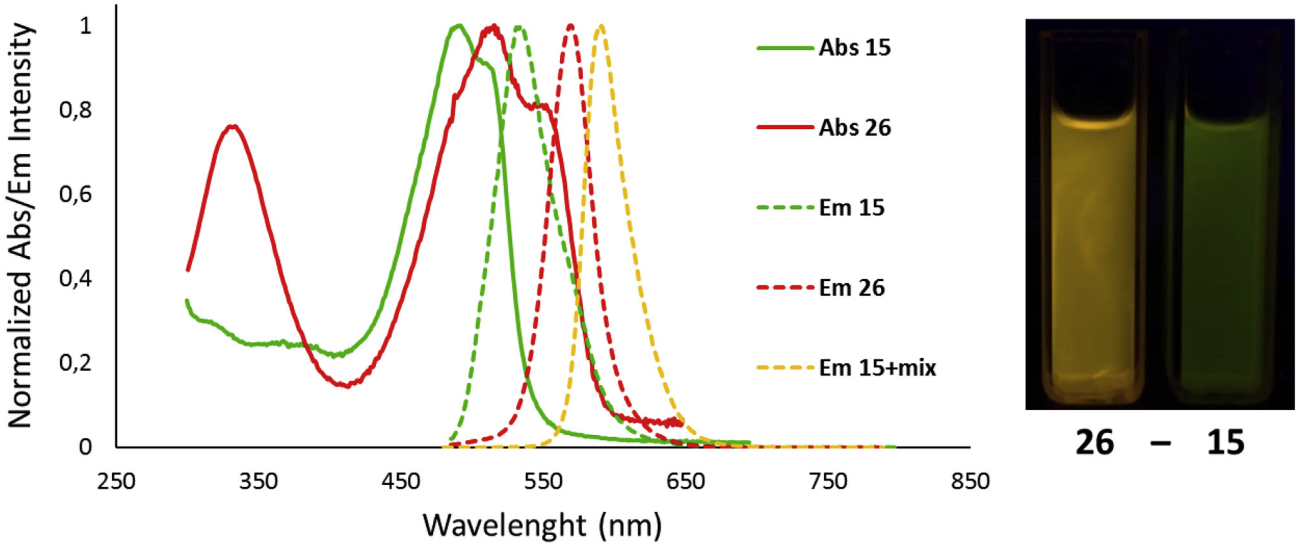


Fig. 3. Absorption and emission spectra (λ_{exc} = 470 nm) fluorescence of compounds **15**, **26** and spectra of emission of mixture of **15** with thiopropanol described in Fig. 1. And emission observed under black light. Conditions: MeOH/H_2O 1:1.

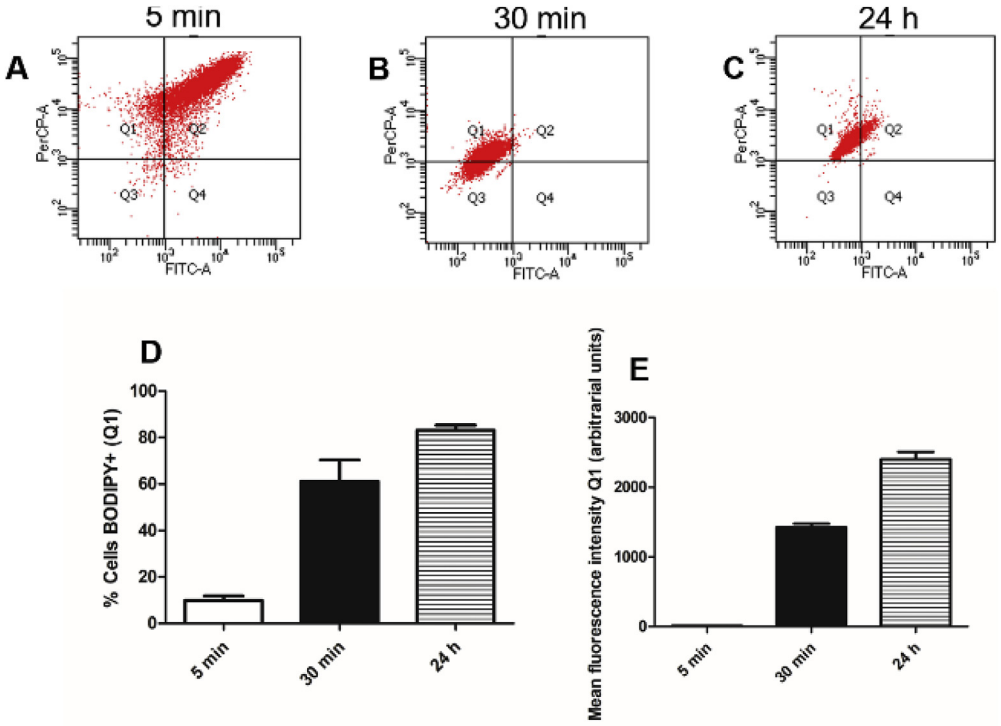


Fig. 4. Flow cytometry studies in tumoral A431 squamous cell carcinoma line after treatment with compound **15** for 5 min, 30 min or 24 h. In Fig. 4A–C, PerCP-A channel λ_{ex} = 488 nm, λ_{em} = 585/42 nm is related to the 3-thio-substituted BODIPYs fluorescence, whereas FITC channel at λ_{ex} = 488 nm, λ_{em} = 530/30 nm is related to 3-nitroso-substituted BODIPYs. Fig. 4D presents the percentage of cells and 4E the fluorescence intensity of 3-thio-substituted BODIPYs. Results are expressed in mean ± SD.

(s, 1H), 7.76–7.70 (m, 3H), 7.68–7.62 (m, 2H), 7.43–7.40 (m, 1H), 7.34 (d, *J* = 4.5 Hz, 1H), 7.06 (dd, *J* = 4.7, 1.6 Hz, 1H), 6.83 (d, *J* = 4.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSOd$_6$): *δ* 156.3, 148.9, 147.9, 137.9, 137.4, 134.1, 132.0, 131.8, 131.0, 128.9, 126.1, 125.5, 115.3. IR (neat): 3138, 3060, 1584, 1525, 1404, 1334, 1304, 1271, 1108, 981, 814, 728. cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{15}H_{10}BF_2N_3O$ [M + K]$^+$ 336.0517, found 336.0529.

4.1.7. 3-Nitrosyl-8-(2,6-dichlorophenyl)-BODIPY **15**

Prepared from BODIPY **13** (164 mg, 0.488 mmol) using the optimized nitrosation reaction in 78% yield (139 mg, 0.380 mmol); m.p 75–80 °C. (C$_6$H$_{14}$/EtOAc 7:3). ^{1}H NMR (300 MHz, CDCl$_3$): *δ* 8.39 (s, 1H), 7.51 (d, *J* = 4.4 Hz, 3H), 7.13 (d, *J* = 4.4 Hz, 1H), 7.00 (d, *J* = 4.6 Hz, 1H), 6.82 (d, *J* = 4.6 Hz, 1H), 6.49 (d, *J* = 4.4 Hz, 1H).^{13}C NMR (75 MHz, CDCl$_3$): *δ* 156.1, 150.8, 142.6, 139.0, 135.0, 135.0, 133.6, 132.3, 129.9, 128.7, 125.1, 124.7, 115.41. IR (neat): 1608, 1521, 1407, 1333, 1306, 1263, 1108, 991 cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{15}H_8BCl_2F_2N_3O$ [M + K]$^+$ 403.9743, found 403.9737.

4.1.8. 2-Nitrosyl-8-phenyl-BODIPY **16**

Prepared from BODIPY **12** (70 mg, 0.261 mmol) using the optimized nitrosation reaction with 19% yield (15 mg, 0.050 mmol); m.p 195–200 °C. (C$_6$H$_{14}$/DCM 7:3). ^{1}H NMR (400 MHz, DMSOd$_6$): *δ* 8.74 (s, 1H), 8.69 (s, 1H), 7.78–7.72 (m, 3H), 7.70–7.63 (m, 2H), 7.41 (d, *J* = 4.5 Hz, 1H), 7.25 (s, 1H), 7.01 (d, *J* = 4.6 Hz, 1H). ^{13}C NMR (101 MHz, CDCl$_3$): *δ* 151.7, 149.6, 141.7, 137.8, 136.2, 135.4, 132.4, 132.0, 131.8, 130.5, 129.07, 122.6, 122.0. IR (neat): 1584, 1562, 1522, 1389, 1364, 1311, 1255, 1221, 1085, 995 cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{15}H_{10}BF_2N_3O$ [M + K]$^+$ 336.0517, found 336.0519.

4.1.9. 3,8-Diphenyl-5-nitrosyl-BODIPY **21**

Prepared from BODIPY **20** (43 mg, 0.124 mmol) using the optimized nitrosation reaction in 73% yield (34 mg, 0.091 mmol); m.p 221–230 °C. (C$_6$H$_{14}$/DCM 7:3). ^{1}H NMR (300 MHz, CDCl$_3$): *δ* 8.07 (m, 2H), 7.68–7.50 (m, 8H), 7.17 (dd, *J* = 5.9, 4.7 Hz, 2H), 6.96 (d, *J* = 4.8 Hz, 1H), 6.62 (d, *J* = 4.4 Hz, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): *δ* 169.0, 149.7, 145.4, 140.4, 136.5, 134.2, 133.0, 132.2, 131.2, 130.7, 130.7, 130.4, 130.4, 130.3, 129.0, 128.8, 126.9, 124.1, 115.4. IR (neat): 1601, 1583, 1502, 1346, 1306, 1118, 1086, 983 cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{21}H_{14}BF_2N_3O$ [M + K]$^+$ 412.0835, found 412.0829.

4.2. General procedure 2: reaction to obtain the compounds 26

To a stirring solution of the BODIPY **15** (137 mg, 0.375 mmol) in methanol (10 mL), thiopropanol (1 eq.) was added, and the reaction course was followed by TLC every 20 min. After full consumption of the starting material, water (15 mL) was added and left stirring for 5 min. The solution is, then, extracted three times with DCM (15 mL), dried over magnesium sulfate and evaporated on rotary evaporator. The residue is purified chromatographically in a silica gel column, using mixtures of Hex:EtOAc as eluents.

4.2.1. 3-Propylthio-5-amine-8-(2,6-dichlorophenyl)-BODIPY **26**

(41 mg, 0.097 mmol) with 26% yield; m.p 90–94 °C. (C$_6$H$_{14}$/EtOAc 1:1). ^{1}H NMR (300 MHz, CDCl$_3$): *δ* 7.45–7.29 (m, 3H), 6.56 (d, *J* = 4.8 Hz, 1H), 6.21 (d, *J* = 3.9 Hz, 1H), 6.16 (d, *J* = 3.9 Hz, 1H), 6.03 (d, *J* = 4.8 Hz, 1H), 5.87 (s, 2H), 2.99 (t, *J* = 7.3 Hz, 2H), 1.78 (h, *J* = 7.4 Hz, 2H), 1.06 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): *δ* 161.0, 136.5, 136.3, 133.2, 132.0, 130.6, 128.3, 128.2, 126.0, 125.6, 120.26, 115.1, 113.4, 36.3, 22.7, 13.6. IR (neat): 3372, 2963, 2929, 2873, 1641, 1603, 1532, 1453, 1423, 1363, 1287, 1171, 1050, 956, 802 cm^{-1}. HRMS (ESI): *m/z* calcd. for $C_{18}H_{17}BCl_2F_2N_3S^+$ [M + H]$^+$ 426.0581, found 426.0575.

4.3. General procedure 3: photophysical and analytical assays

Absorption and emission spectra were recorded on a UV/vis spectrophotometer and on a fluorimeter (λ_{exc} 470 nm), respectively. Fluorescence quantum yields were calculated using a comparative method with a fluorescein standard (0.1 M in NaOH (aq.) – Φ = 0.91, λ_{exc} = 470 nm). The integrated fluorescences of five diluted samples were recorded and plotted against the absorbance. The slope of each linear tendency curve was calculated and the quantum yield of the tested compound (Φ_x) was obtained, using the following equation:

$$\Phi_x = \Phi_{st}\left[\frac{m_x}{m_{st}}\right]\left[\frac{n_x}{n_{st}}\right]^2$$

where Φ_{st} is the quantum yield of the standard, m_x and m_{st} are the slopes for the test compound and the standard compound, respectively, and n_x and n_{st} are the refractive indices of the solvents.

To access the potential analytical application of nitroso-BODIPYs, 1 mL solutions of several analytes in distilled water (50 mM) were prepared. Besides the 20 standard amino acids, aqueous solutions of ethylamine, thiopropanol, 2-mercaptopyridine, cystine, *N*-acetylcysteine and glutathione were also prepared. Each solution was treated with 1 mL of a methanolic solution of the BODIPY **15** (0.5 mM), and fluorescence spectrum was recorded after stirring with a magnetic stirrer for 1 min. Fluorescence emission under black light (370 nm) incidence was also recorded via digital photography. Besides compound **15**, other nitrosylated compounds were also tested in a similar manner with specific analytes.

4.4. General procedure 4: cellular uptake

A431 human squamous cell carcinoma cell line was cultivated in DMEM, containing 10% heat-inactivated FBS and 1% (v/v) of an antibiotic/antimycotic solution (10.000 IU of penicillin, 10 mg of streptomycin and 25 μg of amphotericin B per mL). The cells were kept at 37 °C in a 5% CO_2 atmosphere. Cellular uptake of BODIPY **15** was analyzed by flow cytometry. For this purpose, tumor cells, at 7×10^5 cells/well, were seeded onto 6-wells microplates, containing sterilized cover slips for 24 h at 37 °C and 5% CO_2. The cells were then rinsed using sterilized saline and incubated with BODIPY (stock solution 1 mg/mL in DMSO diluted 1:2500 using culture medium) during 5 min, 30 min and 24 h (n = 3 per incubation time). Flow cytometry analysis was performed, after rinsing the treated cells, using saline and trypsin at 0.25%, followed by neutralization using complete DMEM medium. The samples' fluorescence was then monitored using flow cytometer (BD FACSCanto I) and 10,000 count at λ_{ex} = 488 nm, λ_{em} = 530/30 nm (FITC channel, 3-nitroso-substituted BODIPY) and λ_{ex} = 488 nm, λ_{em} = 585/42 nm (PerCP-A channel, 3-thio-substituted BODIPY). Treatment controls using culture medium without BODIPY were performed at each time point and cellular viability was monitored with or without BODIPY treatment. By the end of the analysis, cell viability was also monitored with 3 μL of propidium iodide (50 μg/mL) at λ_{ex} = 488 nm, λ_{em} = 530/30 nm. Statistical analysis was performed, using One-way ANOVA with Tukey's multiple comparison test and a level of significance set at 0.05.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dyepig.2019.107885.

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