Photochemical formation of BODIPY-quinone methides: spectroscopic study and biological applications

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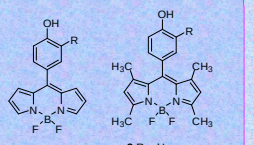
INTRODUCTION

Quinone methides (QMs) are reactive intermediates in the photochemistry of phenols that have attracted scientific interest owing to their biological activity.[1] Moreover, simple QM precursors which generate QMs in photochemical reactions represent a potential class of compounds that could be developed as photoactivable drugs to treat cancer.[2] We have studied photochemical reactions of different classes of phenol molecules that undergo dehydration or deamination and deliver QMs, and investigated antiproliferative activity which QMs induce upon photogeneration.[2] Recently we have found an example of anthrol molecules that can be excited at 405 nm and deliver QMs, which exhibit selective cytotoxic effect on cancer stem cells.[3] However, for the real application in biology or medicine it is pivotal to develop new molecules that can be excited at wavelengths higher than 650 nm. Therefore, we have incorporated QM precursors to BODIPY chromophores.[4] Interestingly BODIPY molecules **2**, **4** and **5** do not undergo deamination from S_1, but they react from higher excited singlet states. The applicability of BODIPY-QMs was investigated by MTT tests on several human cancer cell lines with and without the irradiation. Furthermore, we investigated the possibility to photochemically stain proteins with BODIPY fluorophores.



1 R = $CH_2N(CH_3)_2$
2 R = $CH_2N(CH_3)_2$xHCl
3 R = H
4 R = $CH_2N(CH_3)_2$
5 R = $CH_2N(CH_3)_2$xHCl

Fluorescence study

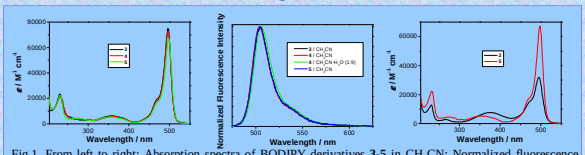


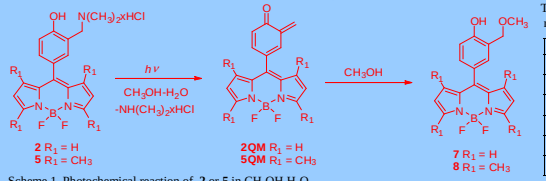
Fig.1. From left to right: Absorption spectra of BODIPY derivatives **3**-**5** in CH_3CN; Normalized fluorescence spectra of **3**-**5** in CH_3CN or CH_3CN-H_2O (1:9) (λ_{ex} = 460 nm); and comparison between absorption spectra of **2** and **5**.

Table 1. Photophysical properties of **2**-**5**.

	Φ (CH_3CN)	Φ (CH_3CN-H_2O)	τ (CH_3CN)/ns [a]	τ (CH_3CN-H_2O)/ns [a]
2	(1.7± 0.3)×10^{-3}	(9.5± 0.5)×10^{-3}	0.01 (13%) 0.13 ± 0.01 (25%) 1.2 ± 0.1 (8%) 11 ± 1 (54%)	0.18 (40%) 3.5 (20%) 15.3 (40%)
3	0.62 ± 0.04	0.11 ± 0.03	3.50 ± 0.02	0.16 ± 0.05 (5%) 0.90 ± 0.05 (95%)
4	0.12 ± 0.02	0.20 ± 0.02	0.04 (7%) 3.39 ± 0.02 (93 %)	0.50 ± 0.03 (5%) 3.54 ± 0.01 (95%)
5	0.013±0.001	0.33 ± 0.03	0.04 (46%) 1.0 ± 0.1 (7%) 3.4 ± 0.2 (47%)	0.13 ± 0.01 (1%) 3.57 ± 0.01 (99%)

[a] Contribution of the decay component is given in parenthesis.

Photochemical reactivity and laser flash photolysis



2 R_1 = H
5 R_1 = CH_3
2QM R_1 = H
5QM R_1 = CH_3
7 R_1 = H
8 R_1 = CH_3

Scheme 1. Photochemical reaction of **2** or **5** in CH_3OH-H_2O.

Table 2. Yields (%) on photoproducts **7** and **8** upon irradiation of **2** and **5**, respectively, at different wavelengths (irradiated for16 h, unless stated otherwise).

Compound/conditions	254 nm	300 nm	350 nm	vis (cool white)
2 / CH_3OH	decomp.	2		N. R.
2 / CH_3OH-H_2O (5:1), pH 7	68	42	2	< 1
2 / CH_3OH-H_2O (5:1), pH 9	decomp.	52	8	< 1
5 / CH_3OH	30 (9h)	12 (12 h)	1	N. R.
5 / CH_3OH-H_2O (5:1), pH 7	100 (2h)	100 (6h)	21	2
5 / CH_3OH-H_2O (5:1), pH 9	94 (4h)	100 (6h)	19	2

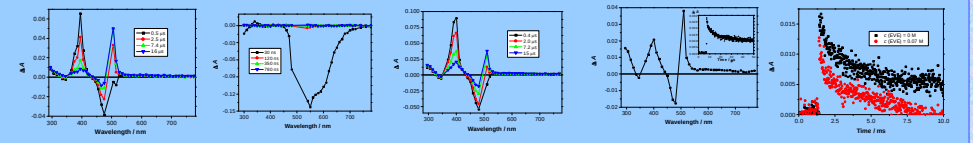


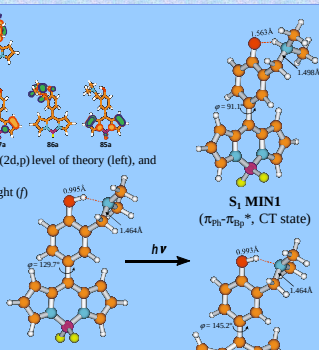
Fig.2. From left to right: Transient absorption spectra of **4** in O_2-purged CH_3CN-H_2O (1:1) solution (λ_{ex} = 355 nm); Transient absorption spectra of **5** in O_2-purged CH_3CN-H_2O (1:1) solution (λ_{ex} = 500 nm); Transient absorption spectra of **5** in O_2-purged CH_3CN-H_2O (1:1) solution (λ_{ex} = 355 nm); Transient spectrum 16 μs after the laser pulse; and decays at 420 nm for **5** in O_2-purged CH_3CN-H_2O (1:1) solution (λ_{ex} = 355 nm). The long lived transient, assigned to **5QM** (lifetime 2.4 ± 0.5 ms) can be quenched by ethyl vinyl ether, k_q = 7 × 10^3 M^{-1} s^{-1}, or NaN_3, k_q = 1 × 10^6 M^{-1} s^{-1}.

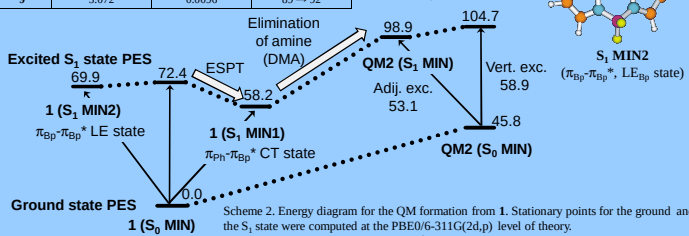
Calculations

Fig. 3. Calculated spectrum of **1** at the PBE0/6-311+G(2d,p)//PBE0/6-311G(2d,p) level of theory (left), and the main orbitals involved in the transitions (right) .

Table 3. TD-DFT calculated vertical exitation energies (E_{exc}), oscilator strenght (f) and leading configurations for **1** (PBE0/6-311+G(2d,p)) .

	E_{exc} / eV	f	Leading conf.
1	3.108	0.3700	89 → 90
2	3.116	0.2365	88 → 90
3	3.674	0.0819	87 → 90
4	3.888	0.0355	85 → 90
7	4.752	0.0077	89 → 91
8	4.939	0.0583	88 → 91
9	5.072	0.0096	89 → 92





Scheme 2. Energy diagram for the QM formation from **1**. Stationary points for the ground and the S_1 state were computed at the PBE0/6-311G(2d,p) level of theory. Relative energies are given in kcal mol^{-1}. Optimized structures (S_0 and S_1) are shown above.

Biology

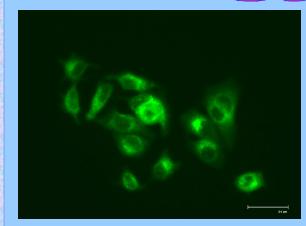
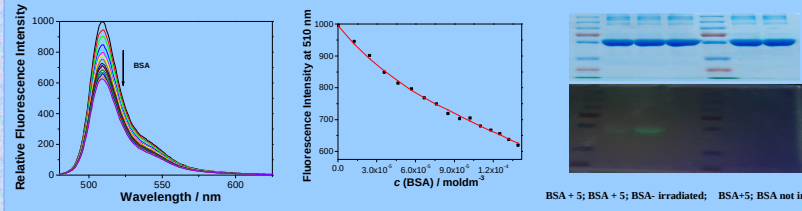


Fig. 4. Image of live MCF-7 cells stained with **5** (c = 10^{-5} M, λ_{exc} = 482/18 nm, λ_{es} = 532/59 nm, Floid® Cell Imagine station).

Table 4. IC_{50} values (in μM) from the MTT test on three human cancer cell lines (MCF-7 breast carcinoma, HCT 116 colon carcinoma and H460 lung carcinoma), with the cells kept in dark or irradiated.

	MCF-7			H460			HCT 116		
	Not irrad.	VIS 3×15 min	300nm 3×1 min	Not irrad.	VIS 3×15 min	300nm 3×1 min	Not irrad.	VIS 3×15 min	300nm 3×1 min
2	11±0.4	13±5	14±6	≥100	53±5	≥100	17±8	12±1	20±3
5	3±2	0.4±0.2	2±1	14±2	1±0.3	13±1	10±0.1	1±0.2	8±1



BSA + 5; BSA + 5; BSA- irradiated; BSA+5; BSA not irrad.

Fig.5. From left to right: Fluorescence spectra (λ_{ex} = 460 nm) of **5** in CH_3CN-H_2O (1:4, phosphate buffer pH = 7.0, c = 0.05 M), with increasing concentration of bovine serum albumin (BSA); Dependence of the fluorescence intensity at 510 nm on the BSA concentration, the fluorescence data was processed by multivariate non-linear regression analysis using model for the formation of a complex **1@BSA** 1:1, logK_a = 5.0 ± 0.1; SDS-PAGE after photoinduced labelling of BSA with compound **5** (20 μg of BSA was incubated without **5**, or with **5** (c = 1 mM), and irradiated at 350 nm (30 min, 8 lamps). The BSA incubated with **5** was then subjected to SDS-PAGE. The labelled BSA was visualised in gel using UV transilluminator- bottom ,or stained with coomassie blue-top).

CONCLUSION

- **BODIPY-phenol 1 deactivates from S_1 by two channels, ESIPT and torsional motion of the *meso*-phenyl group, leading to two minima on the S_1 surface. A minimum on the S_1 surface for BODIPY 2 could not be found computationaly on the TD-DFT level, probably due to existence of conical intersections in vicintiy to the FC state.**
- **Deamination does not take place from 1, 2, 4 or 5 upon excitation to S_1 due to an energy barrier. However, upon excitation to higher singlet states, particularly those that are located on the phenol moiety, the molecule has enough energy for the deamination to take place leading to the formation of QMs.**
- **5QM was detected by LFP (λ_{max} = 395 and 505 nm, τ = 2.4 ms), assignment proved by quenching experiments.**
- **BODIPY derivatives enter cells and induce antiprolifearive effect that is enhanced upon irradiation with visible light (probably due to the formation of singlet oxygen).**
- **BODIPY compounds bind to BSA by non-covalent interactions (log K ≈ 5), whereas photoexcitation to higher singlet states leads to covalent protein modification.**

References

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[3] Uzelac, L.; Škalamera, Đ.; Mlinarić-Majerski, K.; Basarić, N.; Kralj, M. *Eur. J. Med. Chem.* **2017**, *137*, 558-574.
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