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Spectroscopy and photophysics of flavin-related compounds: 3-benzyl-lumiflavin

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Molecular structure, spectroscopic and photophysical data for the singlet state of 3-benzyl-lumiflavin in different solvents are presented. Theoretical studies concerning singlet–singlet and triplet–triplet excitation energies were carried out using time-dependent density functional theory (TD-DFT) calculations. These predictions are in good agreement with the experimental results, which reflect the solvent interactions. All the observable singlet–singlet transitions have π – π^* character. The title compound appears to be an efficient sensitizer of the production of singlet oxygen ($\phi_{\Delta} = 0.53$). The crystal structure of 3-benzyl-lumiflavin is also presented, along with its solid-state photophysical data.

Introduction

Flavins are compounds of considerable interest due to their biological function. Since it has become clear that they are involved in numerous biological processes they have become an object of very intensive studies. Flavins emit characteristic fluorescence with the emission wavelength at about 500 nm, which makes them easy to detect in live organisms. Lumiflavin (7,8,10-trimethyl-10H-benzo[g]pteridine-2,4-dione) is the parent molecule of many flavins, e.g. riboflavin (vitamin B₂), flavin mononucleotide (FMN), and flavin adenine dinucleotide (FAD). We wish to refer to the symposium series titled *Flavins and Flavoproteins*,¹ which shows the wealth of the available information, as well as the progress in the investigation of the photochemistry, structure and functionality of flavins.

In contrast to our previous work, devoted mostly to molecules of alloxazine-type structure, recently we have focused on the nature of the spectral and photophysical properties of flavins. Lately we have described the photophysical properties of lumiflavin and its derivatives modified at the position 3 of the lumiflavin ring by an alkyl substituent, namely 3-methyl and 3-ethyl derivatives, and a number of isoalloxazines with a methyl group substituent at different positions.^{2–7} These compounds have remained in the sphere of our interest for the last few years because of their interesting properties in both singlet and triplet excited states. We would especially like to investigate their role as photosensitizers in singlet oxygen production.

The present work describes processes occurring in singlet and triplet excited states of 3-benzyl-lumiflavin in solution using experimental results and theoretical predictions on the basis of time-dependent density functional theory (TD-DFT).⁸ This 3-benzyl derivative was chosen in order to inquire whether a large aromatic substituent would affect spectral and photophysical properties to a significant extent. The information available on 3-benzyl-lumiflavin is very limited. To the best of our knowledge

there is no published information on its photophysical properties. Fig. 1 presents the molecular structure of both the title compound and its parent molecule, lumiflavin.

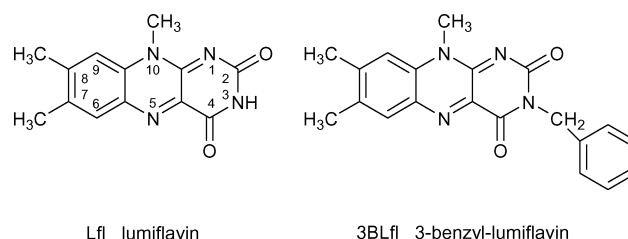


Fig. 1 Molecular structures of lumiflavin and 3-benzyl-lumiflavin.

Experimental

Spectral and photophysical measurements

Methanol and other solvents, of spectroscopic or HPLC grades (Aldrich, Merck) were used as received. The purity of the solvents was confirmed by the absence of fluorescence at the maximum sensitivity of the spectrofluorometer. 3-Benzyl-lumiflavin was a gift from Professor A. Koziołowa. ¹H NMR (CD₃OD) δ : 7.95 (s, 1H, C₆-H), 7.77 (s, 1H, C₉-H), 7.45 (dd, 2H, $J = 6.6$ Hz, C₃₃-H, C₃₇-H), 7.25 (m, 3H, C₃₄-H, C₃₅-H, C₃₆-H), 5.23 (s, 2H, C₃₁-H), 4.12 (s, 3H, C₁₀₁-H), 2.57 (s, 3H, C₈₁-H), 2.46 (s, 3H, C₇₁-H). ¹³C NMR (CD₃OD) δ : 160.66 (C₄), 150.96 (C₂), 149.41 (C_{10a}), 148.36 (C₈), 137.21 (C₃₂), 137.13 (C_{4a}), 135.64 (C₇), 134.89 (C_{5a}), 131.05 (C₆), 128.99 (C_{9a}), 128.08 (C₃₃, C₃₇), 127.97 (C₃₄, C₃₆), 127.05 (C₃₅), 116.05 (C₉), 44.65 (C₃₁), 31.37 (C₁₀₁), 19.87 (C₈₁), 17.97 (C₇₁).

All experiments were carried out at room temperature. UV-Vis absorption spectra were recorded on a Varian Cary 5E

spectrophotometer. Steady-state fluorescence spectra were recorded on a Jobin Yvon-Spex Fluorolog 3-11 spectrofluorometer. Fluorescence quantum yields were determined using quinine sulfate in 0.1 M H₂SO₄ as a standard ($\phi_F = 0.52$). Fluorescence lifetimes were measured using excitation at 450 nm and single photon timing technique on an IBH model 5000U instrument.

Transient absorption measurements were performed by using a nanosecond laser flash photolysis system available in Barcelona, with right-angle geometry. The LKS60 instrument from Applied Photophysics was used, employing the third harmonic (355 nm) of a Q-switched Nd:YAG laser (Spectron laser system, UK; pulse width *ca.* 9 ns) for the laser flash excitation.

Laser induced fluorescence emission measurements of the powdered crystalline samples were performed at room temperature, in the front-face arrangement using a system available in Lisbon. A diagram of the system is presented in ref. 9. The system uses 337.1 nm radiation (suitable for lumichrome excitation) of a N₂ laser (Photon Technology Instruments, Model 2000, *ca.* 600 ps FWHM, ~ 1.3 mJ pulse⁻¹) as the excitation source. The resulting emission is collected by a collimating beam probe coupled to a fused silica optical fiber and detected by a gated intensified charge coupled device (ICCD, Oriel model Instaspec V). The ICCD is coupled to a fixed imaging compact spectrograph (Oriel, model FICS 77441). The system can be used either by integrating all light emitted by the sample or in the time-resolved mode by using a delay generator (Stanford Research Systems, model D6535) with a suitable gate width. The ICCD has high-speed (2.2 ns) gating electronics and an intensifier, and covers the 200–900 nm spectral range. Time-resolved absorption and emission spectra are available in the nanosecond to second time range.^{9–11}

Singlet oxygen luminescence experiments were performed by excitation of the sample with the third harmonic (355 nm) of a Nd:YAG laser (Lumonics hyperYAG HY200, 4 mJ pulse⁻¹, 8 ns FWHM). The excitation energy was attenuated by using solutions of sodium nitrite in water. Detection was obtained on an EO-980P liquid nitrogen cooled germanium photodiode detector (North Coast Scientific), with a 1270 nm interference filter (Melles Griot) interposed between sample and detector in order to reduce detection of laser scatter and sensitizer emission, and to isolate the singlet oxygen phosphorescence. Data capture was with a 250 MS s⁻¹ digitizing oscilloscope (Tektronix 2432A) and Microcal Origin was used for data analysis. Perinaphthenone (Aldrich) was used as a reference standard: $\phi_A = 0.95 \pm 0.05$ independent of solvent.¹²

TD-DFT calculations

The results concerning the electronic structure and geometry of 3-benzyl-lumiflavin were obtained using quantum-chemical calculations by means of the density functional theory (DFT).⁸ The calculations were performed using the B3LYP functional¹³ in conjunction with modest 6-31G(d) and 6-311G(d,p) split-valence polarized basis sets, and also using the polarizable continuum model (PCM) within the B3LYP/6-31G(d) setting to include solvent effects.¹⁴ Excitation energies and oscillator strengths in the dipole length representation were calculated for the optimized ground state geometries using the time-dependent (TD) approach as implemented in the Gaussian 03 package of *ab initio* programs.¹⁵ The lowest-energy singlet–singlet transitions, S₀→S_i, have been calculated for the ground state geometry. The excitation energies computed at the B3LYP/6-31G(d) level of theory are estimated to be accurate within 2000–3000 cm⁻¹, usually requiring a shift towards the red to reproduce the experimental spectra. In the present work T₁→T_i excitation energies and transition intensities were determined for the optimized geometry of the lowest triplet state (T₁). We used the unrestricted UB3LYP approach in calculations of the T₁→T_i spectra.

Table 1 Crystal structure and structure refinement parameters of 3-benzyl-lumiflavin

Chemical formula	C ₂₀ H ₁₈ N ₄ O ₂
Formula weight	346.38
Crystal system	Monoclinic
Space group	<i>P</i> 2 ₁ / <i>c</i>
<i>a</i> /Å	7.1730(4)
<i>b</i> /Å	10.2690(5)
<i>c</i> /Å	22.5120(11)
β /°	90.570(4)
<i>V</i> /Å ³	1658.14(15)
<i>Z</i>	4
μ /mm ⁻¹	0.093
Reflections collected, independent [<i>R</i> _{int}]	15642, 4336 [0.031]
<i>R</i> [<i>I</i> > 2σ(<i>I</i>)]	0.047
<i>wR</i> 2 (all data)	0.135

X-Ray diffraction analysis

A colourless plate-like crystal of 3-benzyl-lumiflavin was analyzed at 100(1) K on an Oxford Diffraction KM4CCD diffractometer with graphite-monochromated Mo-K α radiation ($\lambda = 0.71073$ Å). The data were collected using the ω -scan technique to a maximum θ value of 30° and corrected for Lorentz and polarization effects. The structure was solved with SHELXS97¹⁶ and refined by the full-matrix least-squares method with SHELXS97.¹⁷ Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were put in idealized positions and refined isotropically using the riding model with *U*_{iso} values set at 1.2 (1.4 for methyl groups) times *U*_{eq} of the appropriate carrier atom.

CCDC reference number 263636.

See <http://www.rsc.org/suppdata/pp/b5/b503898g/> for crystallographic data in CIF or other electronic format.

Results and discussion

As mentioned in the introduction, the knowledge about 3-benzyl-lumiflavin is very limited. Therefore, we decided to investigate its structure by X-ray diffraction. Crystallographic data of 3-benzyl-lumiflavin are summarized in Table 1. Fig. 2 shows an ORTEP drawing of 3-benzyl-lumiflavin with the numbering scheme. Molecular dimensions are well within the typical values. The three-ring skeleton of the lumiflavin moiety is approximately—but not strictly—planar. The maximum deviation from the least-squares plane calculated through 14 atoms is 0.100(1) Å, and is larger than in similar compounds (for example, 3-methyl-lumiflavin: 0.019 Å¹⁸; lumiflavin–bis(naphthalene-2,3-diol) molecular complex: 0.081 Å¹⁹), and the dihedral angle

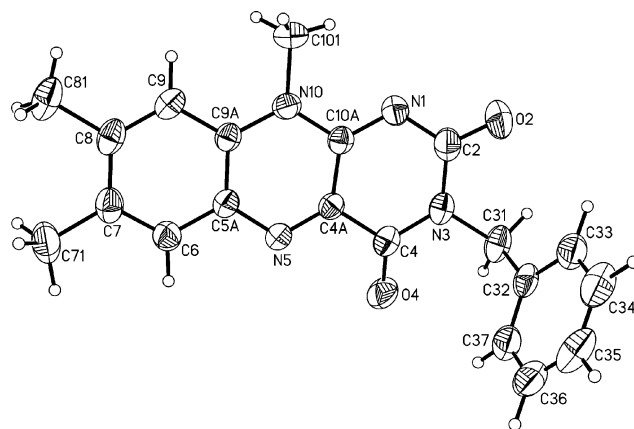


Fig. 2 Anisotropic-ellipsoid representation of 3-benzyl-lumiflavin together with the numbering scheme. The ellipsoids are drawn at 50% probability level, hydrogen atoms are represented by spheres of arbitrary radii.

between the planes of the terminal rings is $4.85(4)^\circ$. The least-squares plane of the phenyl ring (maximum deviation $0.019(1)$ Å) is almost perpendicular to the mean plane of the lumiflavin fragment (dihedral angle $82.02(3)^\circ$). The molecular dimensions are close to those found in similar compounds.^{18,19}

The crystal packing is mainly determined by the van der Waals interactions and by π -stacking interactions between the lumiflavin moieties (Fig. 3). The mean distances between the neighbouring planes in the stack are 3.34 Å and 3.57 Å.

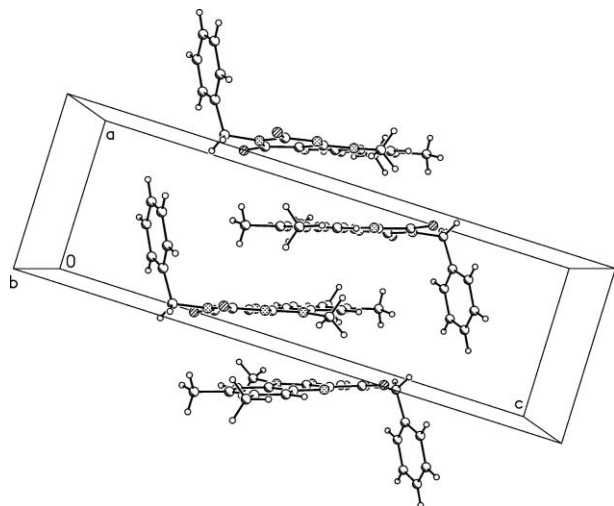


Fig. 3 A fragment of crystal packing, as seen approximately along the [010] direction, showing the π -stacking interaction.

The absorption spectrum of 3-benzyl-lumiflavin in methanol exhibits several bands in the UV-Vis region. In the UV range two intense absorption bands appear at about 225 nm (*ca.* 44.4×10^3 cm⁻¹) and 275 nm (*ca.* 36.3×10^3 cm⁻¹), and one less intense band in the near-UV range at about 350 nm (*ca.* 28.5×10^3 cm⁻¹), see Fig. 4. One more absorption band appears in the visible with the maximum at approximately 450 nm (22.2×10^3 cm⁻¹). 3-Benzyl-lumiflavin emits fluorescence at room temperature. The fluorescence excitation and absorption spectra are in good agreement with each other. The fluorescence emission (excited at 450 nm) is characterized by a single structureless band with a maximum at 534 nm (18.7×10^3 cm⁻¹) and whose position and intensity depend slightly on the solvent, the results are not shown. The fluorescence decays can be satisfactorily described by a single-exponential function in the nanosecond time scale, as confirmed by the usual statistical “goodness-of-fit” criteria. The molar absorption coefficients and other spectral and photophysical data presently obtained are shown in Table 2. The values of the rate constants for the radiative (k_r) and non-radiative ($\sum k_{nr}$) decay for the lowest excited singlet state were calculated as $k_r = \phi_F/\tau_F$ and $\sum k_{nr} = (1 - \phi_F)/\tau_F$, respectively. The data for other lumiflavin derivatives are presented for comparison, indicating that all their photophysical characteristics are almost identical. Therefore, we conclude that the presence of a substituent at position 3 and its character do not affect the photophysics of the parent molecule lumiflavin.

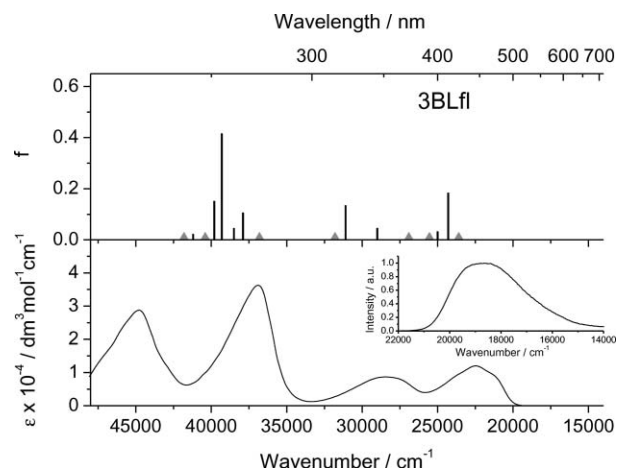


Fig. 4 Predicted lowest-energy singlet-singlet transitions of 3-benzyl-lumiflavin compared to the experimental spectra. Triangles mark the weak $n-\pi^*$ transitions. The experimental ground state absorption spectrum refers to 3-benzyl-lumiflavin in methanol. Inset: fluorescence emission spectrum of 3-benzyl-lumiflavin in methanol.

This result is important when we consider the binding of the lumiflavin molecule in systems of biological interest. We also present the experimental absorption spectrum, (Fig. 4), in comparison with the lowest-energy calculated singlet-singlet transitions scaled according to their oscillator strengths.

According to previous studies, hydrogen bonding by solvent to N(3)-H can influence the electronic structure of the lumiflavin, causing phenomena such as modulation of the reduction potential of flavin to the radical anion.²⁰ In order to prevent any such interactions we have examined lumiflavin derivatives having the 3-alkyl and 3-methyl substituents.²⁻⁶ Our present purpose is to study the lumiflavin derivative with the 3-benzyl substituent. Comparing all the 3-substituted derivatives (3-methyl, 3-ethyl and 3-benzyl-lumiflavin) and lumiflavin, we note that the spectral differences are insignificant. The two absorption bands, appearing in the absorption spectrum of 3-benzyl-lumiflavin at about 450 and 350 nm (*ca.* 22.2×10^3 and 28.6×10^3 cm⁻¹), can be attributed to $\pi-\pi^*$ transitions (see Fig. 4).

Comparing the predicted lowest-energy singlet-singlet transitions of 3-benzyl-lumiflavin and the experimental spectrum (Fig. 4), we can see that the difference between calculated and observed energy values is about 2.0×10^3 cm⁻¹. This fact can be explained by the solvent interactions, which are disregarded in the theoretical calculations. The energy difference is indeed within the admissible range, as detailed in refs. 21 and 22. Moreover, according to the data shown in Table 3, there exist two additional $\pi-\pi^*$ transitions of low oscillator strength, which cannot be seen in the experimental spectrum, at 400, and 344 nm (25.0 , and 29.0×10^3 cm⁻¹).

In 3-benzyl-lumiflavin molecules, $\pi-\pi^*$ transitions are accompanied by $n-\pi^*$ transitions, appearing at about 424, 391, and 371 nm (23.6 , 25.5 , and 26.9×10^3 cm⁻¹) and characterized by low oscillator strengths. These closely located, low-lying excited

Table 2 Spectroscopic and photophysical data for the singlet state of different lumiflavins in methanol^a

Compound	λ_2 /nm	λ_1 /nm	λ_F /nm	ϕ_F	τ_F /ns	$k_r/10^8$ s ⁻¹	$\sum k_{nr}/10^8$ s ⁻¹
3-Benzyl-lumiflavin	353	448 (13000)	534	0.10	5.8	0.17	1.6
3-Ethyl-lumiflavin ^b	350	446 (13000)	532	0.11	6.3	0.17	1.4
3-Methyl-lumiflavin ^c	351	444 (9900)	533	0.15	6.3	0.24	1.3
Lumiflavin ^c	351	442 (12200)	531	0.13	6.8	0.19	1.3

^a λ_1 , λ_2 are the positions of the two lowest-energy bands in the absorption spectra, with the extinction coefficients (in mol⁻¹ dm³ cm⁻¹) given in parentheses, λ_F the fluorescence emission maximum, ϕ_F the fluorescence quantum yield, τ_F the fluorescence lifetime, k_r the radiative rate constant and $\sum k_{nr}$ the sum of non-radiative rate constants. The estimated relative error of ϕ_F and τ_F is 10%. ^b Data from ref. 2. ^c Data from ref. 4.

Table 3 Predicted (B3LYP/6-31G(d)) singlet ($S_0 \rightarrow S_i$) and triplet ($S_0 \rightarrow T_i$) excitation energies starting from the ground state and calculated (UB3LYP/6-31G(d)) triplet ($T_1 \rightarrow T_i$) excitation energies starting from the lowest triplet state of 3-benzyl-lumiflavin, with their corresponding oscillator strengths, f^a

$S_0 \rightarrow S_i$	$E/10^{-3} \text{ cm}^{-1}$	f	$S_0 \rightarrow T_i$	$E/10^{-3} \text{ cm}^{-1}$	f	$T_1 \rightarrow T_i$	$E/10^{-3} \text{ cm}^{-1}$	f
$^1(n, \pi^*)$	23.6	0.001	$^3(\pi, \pi^*)$	16.7	0	$\rightarrow T_2$	7.2	<0.001
$^1(\pi, \pi^*)$	24.3	0.183	$^3(n, \pi^*)$	21.7	0	$\rightarrow T_3$	8.1	0.005
$^1(\pi, \pi^*)$	25.0	0.032	$^3(\pi, \pi^*)$	22.9	0	$\rightarrow T_4$	8.3	<0.001
$^1(n, \pi^*)$	25.5	<0.001	$^3(n, \pi^*)$	23.8	0	$\rightarrow T_5$	8.7	0.003
$^1(n, \pi^*)$	26.9	0.003	$^3(\pi, \pi^*)$	24.1	0	$\rightarrow T_6$	9.3	0.002
$^1(\pi, \pi^*)$	29.0	0.045				$\rightarrow T_7$	10.7	0.003
$^1(\pi, \pi^*)$	31.1	0.134				$\rightarrow T_8$	14.4	0.004
$^1(n, \pi^*)$	31.8	0.002				$\rightarrow T_9$	15.5	0.026
$^1(n, \pi^*)$	36.8	<0.001				$\rightarrow T_{10}$	17.9	0.001
$^1(\pi, \pi^*)$	37.9	0.105				$\rightarrow T_{11}$	18.5	0.129
$^1(\pi, \pi^*)$	38.5	0.045				$\rightarrow T_{12}$	23.2	0.022
$^1(\pi, \pi^*)$	39.3	0.415				$\rightarrow T_{13}$	24.2	0.042
$^1(\pi, \pi^*)$	39.8	0.151				$\rightarrow T_{14}$	26.1	0.001
$^1(n, \pi^*)$	40.4	0.003				$\rightarrow T_{15}$	28.3	0.241
$^1(\pi, \pi^*)$	41.2	0.022				$\rightarrow T_{16}$	28.6	0.002

^a The energy of the lowest triplet state is $15.7 \times 10^3 \text{ cm}^{-1}$ above the ground state.

Table 4 Predicted (6-31G(d)) and (B3LYP/6-311G(d,p)) singlet ($S_0 \rightarrow S_i$) excitation energies of 3-benzyl-lumiflavin starting from the ground state, compared to the same energies calculated taking into account the solvent (MeOH) by virtue of the polarizable continuum model (B3LYP/6-31G(d)), with their corresponding oscillator strengths, f

Isolated 6-31G(d)			Isolated 6-311G(d,p)			In MeOH 6-31G(d)		
$S_0 \rightarrow S_i$	$E/10^{-3} \text{ cm}^{-1}$	f	$S_0 \rightarrow S_i$	$E/10^{-3} \text{ cm}^{-1}$	f	$S_0 \rightarrow S_i$	$E/10^{-3} \text{ cm}^{-1}$	f
$^1(n, \pi^*)$	23.6	0.001	$^1(n, \pi^*)$	23.7	0.005	$^1(\pi, \pi^*)$	24.0	0.175
$^1(\pi, \pi^*)$	24.3	0.183	$^1(\pi, \pi^*)$	24.2	0.201	$^1(n, \pi^*)$	25.8	0.007
$^1(\pi, \pi^*)$	25.0	0.032	$^1(\pi, \pi^*)$	25.1	0.018	$^1(n, \pi^*)$	27.4	<0.001
$^1(n, \pi^*)$	25.5	<0.001	$^1(n, \pi^*)$	25.7	<0.001	$^1(\pi, \pi^*)$	28.2	0.010
$^1(n, \pi^*)$	26.9	0.003	$^1(n, \pi^*)$	26.9	0.003	$^1(n, \pi^*)$	29.0	<0.001
$^1(\pi, \pi^*)$	29.0	0.045	$^1(\pi, \pi^*)$	29.1	0.051	$^1(\pi, \pi^*)$	29.3	0.232
$^1(\pi, \pi^*)$	31.1	0.134	$^1(\pi, \pi^*)$	31.0	0.135	$^1(n, \pi^*)$	31.7	0.005

singlet states of π, π^* and n, π^* character, with the lowest excited singlet state of n, π^* character, differ from those observed in other isoalloxazine derivatives, such as lumiflavin, 3-methyl-lumiflavin and 3-ethyl-lumiflavin, in which the lowest excited singlet state has π, π^* character. However, the energy difference, ΔE , between the lowest n, π^* and π, π^* states is about $0.7 \times 10^3 \text{ cm}^{-1}$ for 3-benzyl-lumiflavin, compared to $0.3 \times 10^3 \text{ cm}^{-1}$ for the other molecules.²⁻⁶ Considering the photophysics and spectroscopy of 3-benzyl-lumiflavin, one could expect the lowest excited singlet to be of π, π^* character, as happens in lumiflavin, and its 3-methyl- and 3-ethyl derivatives. We performed additional calculations using the 6-311G(d,p) basis set in order to verify if that would improve the results. We found that the inclusion of the polarization p functions for the hydrogen atoms and application of a larger basis set did not affect the results significantly. In particular, comparing the lowest-energy singlet-singlet transitions of 3-benzyl-lumiflavin predicted using 6-31(d) and 6-311(d,p) basis sets (see Table 4), we can see that the difference between the two is only about $0.1 \times 10^3 \text{ cm}^{-1}$, with a more pronounced difference between the oscillator strengths. However, the solvent (MeOH) included in the calculations by virtue of the PCM, produced significant changes in the predicted singlet ($S_0 \rightarrow S_i$) excitation energies of 3-benzyl-lumiflavin. First of all, PCM calculations produced order changes between some of the π, π^* and n, π^* transitions. The results obtained with the 6-31G(d) basis set predict the lowest state to be n, π^* with an energy of about $23.6 \times 10^3 \text{ cm}^{-1}$, whereas the PCM data have the lowest transition of π, π^* character, with an energy of *ca.* $24.0 \times 10^3 \text{ cm}^{-1}$. Therefore, the energy difference between the lowest singlet states predicted for 3-benzyl-lumiflavin as an isolated molecule and in methanol solvent, accounted for by the PCM, is only about $0.4 \times 10^3 \text{ cm}^{-1}$. However, much larger changes may be noted comparing the energies of the two lowest π, π^* and n, π^* transitions in the two types of calculations. In fact,

the largest difference of *ca.* $2.2 \times 10^3 \text{ cm}^{-1}$ is observed between the two lowest n, π^* transitions: $23.6 \times 10^3 \text{ cm}^{-1}$ for an isolated molecule, and $25.8 \times 10^3 \text{ cm}^{-1}$ in methanol solvent. The energy of the π, π^* transition is less affected by the solvent, changing from $24.3 \times 10^3 \text{ cm}^{-1}$ for the isolated molecule to $24.0 \times 10^3 \text{ cm}^{-1}$ in methanol solvent.

The electronic structure of 3-benzyl-lumiflavin in the triplet state was described using the experimental T-T absorption spectrum in comparison with the theoretical DFT results (Fig. 5). The T-T excitation energies and transition intensities in

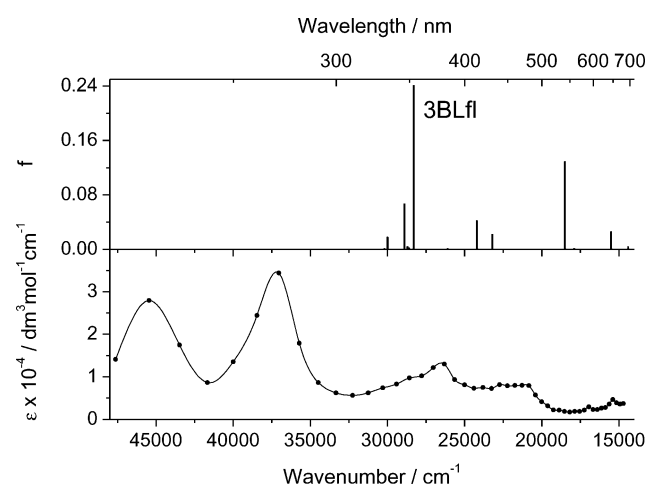


Fig. 5 Predicted T-T transitions of 3-benzyl-lumiflavin obtained using the DFT method compared to the experiment. The experimental T-T absorption spectrum was calculated from the transient absorption of 3-benzyl-lumiflavin in methanol excited at 355 nm, assuming the $1.3 \times 10^4 \text{ mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$ triplet extinction coefficient value at 380 nm, typical for other lumiflavin derivatives.

DFT calculations were determined for the optimized geometry of the lowest triplet state (T_1), with the results shown in Table 3. Experimental data, obtained from the transient absorption spectrum, exhibit bands at 15.4, 16.9, 20.8, 26.3, 37.0, and $45.5 \times 10^3 \text{ cm}^{-1}$. The bands predicted by DFT calculations located at about 15.5, 18.5, 23.2, 24.2, and $28.3 \times 10^3 \text{ cm}^{-1}$ are blue-shifted relative to the corresponding experimental bands. One of the reasons could be the relatively small basis set; however, the increase of the basis set would cause a significant increase of the computation time, and the associated costs, making the calculations impractical. It is usually difficult to describe triplet states using a single determinant. However, application of multi-configuration methods such as MCSCF or CASPT2, to calculations of the UV-Vis, T-T and S-T electronic spectra in such large molecules is beyond the current capabilities of software and available computers. Another reason for the blue shift is the fact that the experimental spectrum was obtained in solution (methanol), while the calculations refer to isolated gas-phase molecules. The solvent effect had been elucidated by comparing the gas-phase uracil spectrum with that in aqueous solution.^{21,22} To the best of our knowledge, there are no published absorption spectra of lumiflavins or related compounds in the gas-phase. However, comparing to the data for uracil,^{21,22} where the gas-phase absorption bands are blue-shifted by about 1200–2500 cm^{-1} relative to the aqueous solution spectrum, we consider the agreement presently obtained between theoretical and experimental data relatively good. Considering the data in Table 3, we note that there exist several lower-energy transitions, which could not be observed in the experimental spectrum, because of the present limitations of the flash-photolysis equipment.

The electronic structure of 3-benzyl-lumiflavin was studied by means of the time-dependent density functional theory (TD-DFT). Fig. 6 depicts the shape of the lowest unoccupied (LUMO) and the highest occupied (HOMO) molecular orbitals, involved in the transitions to the low-lying excited states. Let us consider the $S_0 \rightarrow S_1$ transition, which has a dominant

contribution from the HOMO-1 \rightarrow LUMO excitation and is assigned as an $n \rightarrow \pi^*$ transition. It is interesting that as a result of this transition, all the charge initially accumulated on the benzyl ring gets redistributed over the lumiflavin backbone, which corresponds to an out-of-plane transition. The experimental absorption band, which represents the $S_0 \rightarrow S_1$ transition, indicates that the transition energy is $22.2 \times 10^3 \text{ cm}^{-1}$ for the first π, π^* singlet excited state, while the value predicted with the use of the TD-DFT method is $24.3 \times 10^3 \text{ cm}^{-1}$ (also a π, π^* state), with a good agreement between the two. Note that the lowest singlet excited state predicted in the polarizable continuum model (B3LYP/6-31G(d)), is of π, π^* type with an energy of $24.3 \times 10^3 \text{ cm}^{-1}$, and has a dominant contribution from the HOMO \rightarrow LUMO excitation.

TD-DFT calculations predict the nature of the molecular orbitals involved in the predominant excitations ($S_0 \rightarrow T_1$), and the symmetry of the corresponding T_i states. According to the data presented in Table 3, Fig. 5, the ($S_0 \rightarrow T_1$) excitation has $^3(\pi, \pi^*)$ character. The dominant contribution is from the HOMO \rightarrow LUMO excitation. The predicted dipole moment of 3-benzyl-lumiflavin is 8.78 D in the ground state, and 8.17 D in the first triplet state.

Recently we have been directing our interest to the interaction between the excited molecules of the studied compound with oxygen. The driving force to determine quantum yields of singlet oxygen production by flavins and their derivatives is the very limited amount of data of this type.²³ For the purpose of our research we measured the emission at 1270 nm, which is highly specific to the $\text{O}_2(^1\Delta_g) \rightarrow \text{O}_2(^3\Sigma_g^-)$ transition under laser excitation at 355 nm of the 3-benzyl-lumiflavin in air-equilibrated methanol. The emission intensity at 1270 nm increased in samples with higher oxygen concentration and was extinguished by bubbling N_2 through the solution for a few minutes. The apparent first-order rate constant of deactivation of 3-benzyl-lumiflavin triplet in methanol is: $k_T = 2.86 \times 10^4 \text{ s}^{-1}$ after N_2 purging, $k_T = 2.35 \times 10^6 \text{ s}^{-1}$ in air-equilibrated solutions, and $k_T = 1.19 \times 10^7 \text{ s}^{-1}$ in oxygen-saturated solutions. The values of the quantum yield and lifetime of singlet oxygen formed by triplet photosensitization were determined by exciting an air-saturated solution of 3-benzyl-lumiflavin. The results are presented in Table 5, where, for comparison, are also given the respective values for lumiflavin and other derivatives substituted in the 3 position of the lumiflavin ring. The emission lifetime values obtained ($\tau_\Delta = 10 \text{ }\mu\text{s}$) are typical for singlet oxygen in methanol.^{24,25}

All the above observations confirm that 3-benzyl-lumiflavin can act as a photosensitizer for the singlet oxygen and that $\text{O}_2(^1\Delta_g)$ is responsible for the emission at 1270 nm. There is also little difference in singlet oxygen quantum yields between lumiflavin and its 3-alkyl and 3-aryl derivatives. The choice of methanol as a solvent was dictated by the necessity to study the role of the hydrogen bonding interactions. Thus it can be concluded that the N(3)-aryl substituent does not influence significantly the interaction between flavin and oxygen. The title lumiflavin derivative can be an efficient source of singlet oxygen and the benzyl substituent does not affect this efficiency.

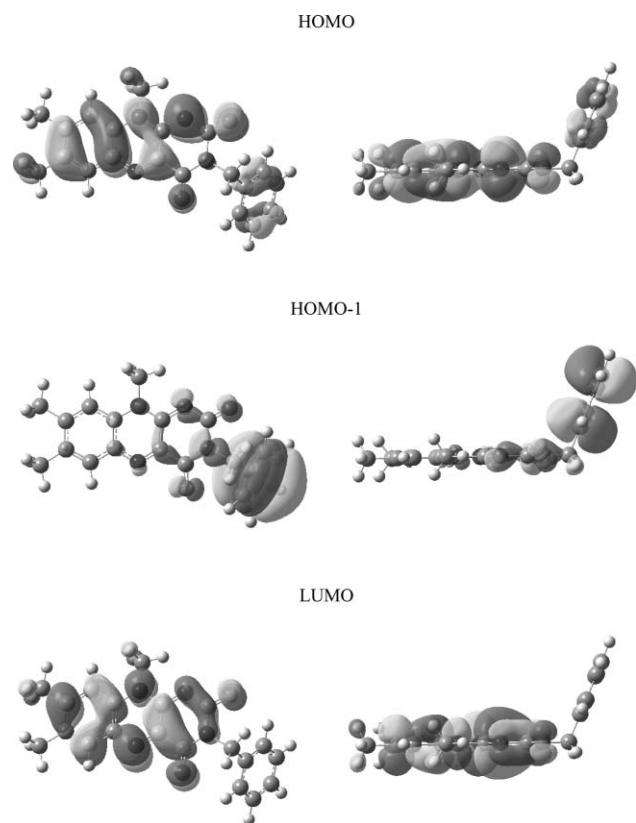


Fig. 6 Shape of the principal molecular orbitals involved in the low-energy transitions in 3-benzyl-lumiflavin. The isosurfaces correspond to the value of ± 0.02 .

Table 5 Triplet state lifetimes, τ_T , quantum yields of photosensitized production of singlet oxygen, ϕ_Δ , and the singlet oxygen lifetimes, τ_Δ , for selected lumiflavin derivatives in methanol solutions

Compound	$\tau_T/\mu\text{s}$	ϕ_Δ	$\tau_\Delta/\mu\text{s}$
Lumiflavin	16.6	0.48 ^a	10 ^a
3-Benzyl-lumiflavin	34.9	0.53	10
3-Methyl-lumiflavin		0.53	10
3-Ethyl-lumiflavin	9.5	0.55 ^a	10 ^a

^a Data from ref. 4.

Ground state diffuse reflectance absorption spectra in the solid state were recorded and plotted using the Kubelka–Munk remission function (see Fig. 7); in addition, we also recorded time-resolved emission spectra of 3-benzyl-lumiflavin polycrystals as illustrated in Fig. 7. The spectral bands are interpreted by analogy to the situation in solution. However, the absorption edge and the emission maximum get shifted to the red, and the fluorescence lifetime becomes shorter in solid 3-benzyl-lumiflavin if compared to solution. Specifically, the emission maximum in the solid occurs at about 582 nm, which is a significant red shift compared to 534 nm in methanol solution. The fluorescence lifetime of the solid sample is about 3.5 ns, and is shorter than the corresponding value in solution, 5.8 ns. All these results suggest the importance of molecular interactions in the crystal packing of 3-benzyl-lumiflavin in the solid state, as evident from our X-ray data. Clearly, further work is needed to clarify the 3-benzyl-lumiflavin photophysics in the solid state.

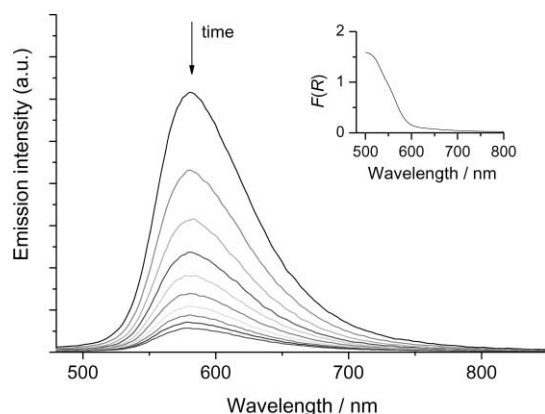


Fig. 7 Time-resolved fluorescence emission spectra of 3-benzyl-lumiflavin polycrystals. Excitation was at 337 nm, spectra recorded with the time step of 1 ns. Insert: ground state diffuse reflectance absorption spectrum of the same polycrystals plotted using the Kubelka–Munk remission function.

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