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ORIGINAL PAPER

# Unusual Light Spectroscopic Properties of a 2-Pyridone-Based Multi-Ring-Fused Fluorescent Scaffold

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**Abstract** UV-VIS absorption and fluorescence spectroscopic properties of six related polyaromatic 2-pyridones have been studied. Excitation of the lowest and rather weak and structure-less transition [$\varepsilon_{max}$ (430 nm)≈ 3,000 $mol^{-1}dm^3cm^{-1}$] gives rise to a broad fluorescence band in the visible region, for these compounds. These $S_0 \leftrightarrow S_1$ transitions are compatible with symmetrically forbidden transitions, promoted by intensity borrowing, as is revealed by fluorescence depolarisation data. With one exception, all compounds exhibit strong fluorescence, with quantum yields in glycerol varying between 40% and 70%. The corresponding fluorescence lifetimes range from 11 ns to 17 ns, while the radiative lifetimes are very similar (≈26 ns), for all compounds. Interestingly and rarely observed, the calculated radiative lifetimes for the weak absorption band are significantly longer, i.e. between 37 and 40 ns.

**Keywords** 2-pyridone derivatives · Fluorescence quantum yield · Fluorescence lifetime · Fluorescence spectrum · Radiative lifetime · Intensity borrowing

## Introduction

Small organic compounds with fluorescent properties have found wide applications in molecular biology, as intracellular probes for calcium ion concentrations, or as organell specific dyes [1]. Recently a series of polyaromatic 2-pyridones were synthesizied and their use as cell-staining fluorescent dyes were demonstrated [2]. There exists many naturally occurring ring-fused 2-pyridones with interesting biological activity, which are described elsewhere, *e. g.* camptotecin [3], and fredericamycin A [4]. The polyaromatic 2-pyridone scaffold can easily be substituted in various ways, potentially allowing it to interact with different biological targets. In the present paper, some of the compounds are found to exhibit unusually long fluorescent lifetimes, and here the absorption and fluorescence spectroscopic properties of these compounds have been more thoroughly examined.

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## Materials & methods

### Chemicals

HPLC-grade dichloromethane, anhydrous glycerol≥99.5% (GC), p.a grade 1,2-propanediol and phloroform, and Spectroscopic grade ethanol was used. All solvents were screened for posessing inherent signals in the spectroscopic range of interest.

### Syntheses of 2-pyridonebased compounds

Compounds of structure **1** (Fig. 1) can be synthesized by cyclocondensation of acyl ketenes and thiazolines [5–8]. The synthesis of the fluorescent polyaromatic compounds **2** is then accomplished in two steps by formylation followed by dehydration [2].

### Absorption and fluorescence spectra

The absorption spectra were recorded on a Varian Cary, 5000 UV-VIS spectrometer. The fluorescence spectra were

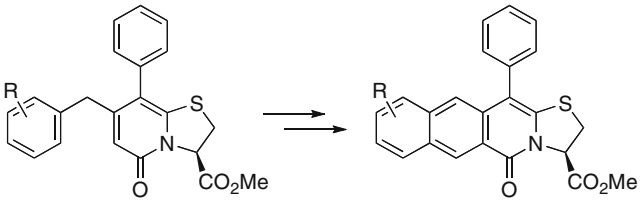


**Fig. 1** Synthesis of the 2-pyridone based polyaromatic compounds

transcribed by means of a Fluorolog® - 3 spectrometer (Jobin Yvon) equipped with Glan-Thompson polarisers. The bandwidth of the excitation and emission light was 2 nm. All fluorescence spectra were corrected.

Fluorescence Depolarisation Measurements

Fluorescence spectra are measured for the following horizontal (H) and vertical (V) polarizer settings: VV, VH, HV and HH. These spectra are used to calculate the fluorescence depolarization r, or anisotropy, according to:

$$r = \frac{F_{VV} - GF_{HV}}{F_{VV} + 2GF_{VH}}$$

where the G-factor is given by:

$$G = \frac{F_{HV}}{F_{HH}}$$

Fluorescence Lifetime Measurements

The time-resolved fluorescence decays were measured by means of the time-correlated single photon-counting technique using a Fluorolog®TCSPC (Horiba) spectrometer. For the pulsed excitation, a NanoLED (Horiba) was used in combination with an interference filter, centred at 410 nm (HBW=11.4 nm). The fluorescence lifetime was calculated by using a deconvolution method [9] based on the Levenberg-Maquardt algorithm [10].

Fluorescence quantum yield

The fluorescence quantum yields ($\Phi_f$) were calculated by comparison with a reference (r) substance, by means of the equation:

$$\Phi_f = \Phi_r \frac{F}{F_r}\left(\frac{1 - 10^{A_r(\lambda_{ex})}}{1 - 10^{A(\lambda_{ex})}}\right)\frac{n^2}{n_r^2}$$

Here, $F$ and $F_{ref}$ is the integrated fluorescence spectrum of the unknown and the reference sample, respectively. $A(\lambda_{ex})$ and $A_{ref}(\lambda_{ex})$ denote the corresponding absorbance at the excitation wavelength ($\lambda_{ex}$), and $n$ is the refractive index for the media in which the substances are dissolved. The used refractive indices were 1.47 (glycerol), 1.43 (1,2-propanediol) and 1.42 (dichloromethane).

The reference substance was $N^2$,$N^3$-[Bis(1-hexylheptyl)-benzo[ghi]perylene-2,3,8,x9,11,12-hexacarboxylic-2,3:8,9:11,12-tris(dicarboximide)]-$N^1$,$N^{1\prime}$-(1,2-ethyl)-[$N^{2\prime}$-(1-octylnonyl)-perylene-3,4:9,10-bis(dicarboximide)] dissolved in dichloro-methane, which has been described elsewhere [11].

The radiative lifetime

The radiative life was calculated from the Strickler-Berg equation [12]:

$$1/\tau_0 = 2.88 \cdot 10^{-9} n^2 \left\langle \tilde{\nu}_f^{-3} \right\rangle^{-1} \int_{band} \frac{\varepsilon(\tilde{\nu})}{\tilde{\nu}} d\tilde{\nu}$$

$$1/\left\langle \tilde{\nu}_f^{-3} \right\rangle = \frac{\int_{band} F(\tilde{\nu}) d\tilde{\nu}}{\int_{band} \frac{F(\tilde{\nu})}{\tilde{\nu}^3} d\tilde{\nu}}$$

where $n$ is the refractive index of the solution, $\tilde{\nu}$ is the wavenumber (in $cm^{-1}$), and $\left\langle \tilde{\nu}_f^{-3} \right\rangle$is the mean value of $\tilde{\nu}^{-3}$ over the emission spectrum, $F(\tilde{\nu})$. The molar absorptivity is in its usual units of $mol^{-1}dm^3cm^{-1}$.

## Results & discussion

Here, the fluorescent properties of six differently substitued compounds, **2a–2f**, are examined. In the series there are both electron withdrawing and electron donating substituents. Compound **2a** is a special case with an extended π-electronic system in contrast to the others. (Fig. 2)

*Absorption and fluorescence spectra* The absorption and fluorescence spectroscopic properties of different polyaromatic 2-pyridones have been studied by using UV-VIS absorption, as well as steady-state and time-resolved fluorescence spectroscopic methods. The rather weak and structureless absorption band in the visible region has a peak molar absorptivity of approximately 3,000 $mol^{-1}dm^3cm^{-1}$, whereas the much stronger transitions in the UV region clearly reveal vibronic structure. With the exception of the 9,10-benzo-fused derivative (**2a**), the absorption and fluorescence spectra of the polyaromatic 2-pyridones exhibit similar shape over an identical wavelength region (cf. Fig. 2). For compounds **2b**–**2f** a vibronic peak is observed at approximately 23,250 $cm^{-1}$ (≈ 430 nm), which for **2a** is shifted to approximately 27,800 $cm^{-1}$ (≈ 430 nm). This spectral red-

| R | $R^2$ | Product |
|---|---|---|
| 9,10-ring-fused benzene | H | **2a** |
| H | H | **2b** |
| H | Methyl | **2c** |
| 10-Methyl | H | **2d** |
| 10-Methoxy | H | **2e** |
| 8-Fluoro | H | **2f** |

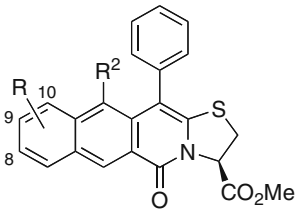


**Fig. 2** The studied polyaromatic 2-pyridone derivatives, denoted 2a–2 f

shift is compatible with the extended $\pi$-electronic system. However, no significant red-shift is revealed from the lowest electronic transition, centred at approximately 25,000 $cm^{-1}$ (≈400 nm), for any of the compounds. The latter is not compatible with symmetry-allowed electronic transitions [13], which will be further discussed in the sub-section "Fluorescence anisotropies". One should note that for the investigated compounds, the absorption spectra cover a broad range, which enables a wide range of useful excitation wavelengths in fluorescence spectroscopic applications.

The fluorescence spectrum centred at *ca.* 21,200 $cm^{-1}$ (470 nm) and *ca*. 20,000 $cm^{-1}$ (500 nm) of **2a** and **2b**–**2f**, respectively, is rather structure-less and covers a major part of the visible region (cf. Fig. 3). The shapes resemble mirror images of the corresponding weak absorption bands.

*Fluorescence anisotropies* The steady-state excitation and emission anisotropies, $r(\tilde{\nu}_{ex})$ and $r(\tilde{\nu}_{em})$, respectively, were determined for the compounds (**2b**–**2f**) when dissolved in glycerol at 269 K (cf. Fig. 4). The influence of reorienting motions is negligible at this temperature. Therefore the obtained data represent the fundamental anisotropy, i.e. $r_0(\tilde{\nu})$ [14]. For **2b**, the region of nearly constant $r(\tilde{\nu}_{ex})$-values reveals two strong absorption bands at approximately 28,000 and 34,500 $cm^{-1}$, corresponding to symmetry-allowed electronic transitions. The negative and similar $r(\tilde{\nu}_{ex})$-values indicate that these electronic transition dipoles tend to be polarised perpendicular to the emission transition dipole. On the other hand, the obtained $r(\tilde{\nu}_{ex})$ for the weak absorption band depends strongly on the excitation energy. This shows that the weak absorption band constitutes multiple transition dipole moments whose directions in the molecular frame depend on the excitation energy.

The magnitude of the determined emission anisotropy also strongly depends on the excitation energy, whereas the $r(\tilde{\nu}_{em})$-levels exhibit weak dependence on $\tilde{\nu}_{em}$. However, in the red edge of the absorption spectrum and blue edge of the fluorescence spectrum, $r(\tilde{\nu}_{ex}) \rightarrow r(\tilde{\nu}_{em}) \approx 0.35$, which means that the electronic transition moments tend to be parallel. Theoretically, for $\pi \leftrightarrow \pi^*$ transitions that correspond to parallel transition dipole moments, $r(\tilde{\nu}_{ex}) = r(\tilde{\nu}_{em}) = 2/5$ [1]. The weak absorption band that shows a varying transition dipole direction is compatible with a symmetry-forbidden electronic transition. Thereby, the dependence of the electronic transition on the nuclear geometry cannot be neglected. By using first-order perturbation theory, it is possible to show that a non-vanishing transition probability is possible though

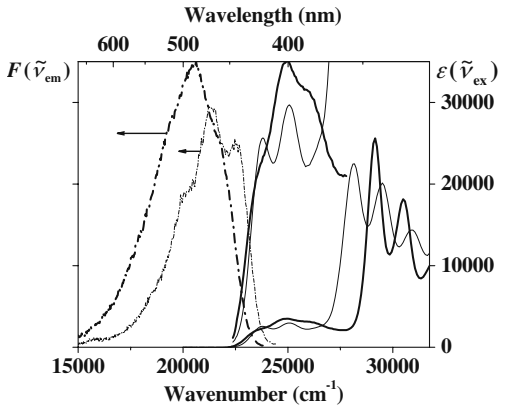


**Fig. 3** The molar absorptivity [$\varepsilon(\tilde{\nu}_{ex})mol^{-1}dm^3cm^{-1}$] of compounds **2a** (*thin line*) and **2b** (*bold line*) is shown in the right hand part of the figure. Inserted and 10 times magnified are the weak absorption bands centred at *ca*. 25,000 $cm^{-1}$. Also displayed is the corrected fluorescence spectrum [$F(\tilde{\nu}_{em})$] of **2a** (*thin dot-dashed line*) and **2b** (*bold dot-dashed line*). The compounds were dissolved in chloroform

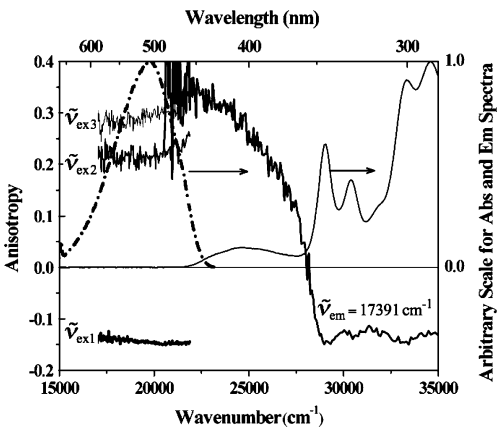


**Fig. 4** Fluorescence (-.-.-.-) and absorption (——) spectra of compound **2b** are displayed together with the excitation anisotropy ($\tilde{\nu}_{em}$ = 17391 $cm^{-1}$) and emission anisotropies recorded at $\tilde{\nu}_{ex1}$ = 30487 $cm^{-1}$, $\tilde{\nu}_{ex2}$ = 26315 $cm^{-1}$ and $\tilde{\nu}_{ex3}$ = 25000 $cm^{-1}$. The depolarisation data of the compound dissolved in glycerol were collected at 269 K

intensity borrowing, from higher electronic states through vibronic mixing [13]. Similar conclusions can be drawn for **2a** by viewing its emission anisotropy spectrum (data not shown).

*Fluorescence quantum yields and lifetimes* The fluorescence quantum yields of compounds **2b**–**2f** are rather high and depend on the solvent (cf. Table 1). Among aromatic compounds the fluorescence lifetimes are unusually long, typically ranging from ~6 ns to 17 ns and also depend on the solvent. The fluorescence decays are single exponential functions. In these respects the compound **2a** differs, with fluorescence lifetimes of 1–2 ns and quantum yields below 10%. From the well-known relation between the fluorescence lifetime ($\tau_f$), the quantum yield ($\Phi_f$) and the radiative lifetime ($\tau_0^{exp}$), i.e. $\tau_f = \Phi_f \tau_0^{exp}$, the latter lifetime can be calculated. The obtained values are summarised in Table 1. For compounds **2b**–**2f** the radiative lifetimes are very similar exhibiting an average value of 25.8 ns in glycerol and 27.7 ns in methylene chloride. The accuracy of the corresponding values for **2a** in glycerol (25.3 ns) and methylene chloride (33 ns) is much less, due to uncertainties in the determination of low quantum yields. The radiative lifetime of a compound dissolved in glycerol tends to be somewhat longer than that in methylene chloride. A rather long radiative lifetime is expected for states corresponding to weak electronic transitions. It is, however, possible to estimate the value of the radiative lifetime ($\tau_0$) by using the Strickler-Berg equation [12]. For the weak absorption band centred at approximately 25 000 $cm^{-1}$, one obtains that $\tau_0$=37.9±1 ns and 38.9±1 ns for **2b** dissolved in glycerol and methylene chloride, respectively. A calculated value of $\tau_0$=57.4±5 ns is obtained for **2a** in chloroform. Hence, the predicted radiative lifetimes are significantly longer than the values determined from the experimental lifetimes and quantum yields. In deriving the Strickler-Berg equation a mirror symmetry relation is assumed between the absorption band of the lowest electronic transition and the fluorescence spectrum. This indicates that the nuclear configurations of the ground ($S_0$) and excited ($S_1$) electronic states are sufficiently similar that the vibrational wavefunctions are the same in $S_0$ and $S_1$. Indeed, the mirror symmetry seems to be fulfilled for compounds **2a**–**2f** (cf. Fig. 3). One reason for the discrepancy could be an underestimation of the molar absorptivity of the compounds, which is the case if the compound is not pure. From the radiative lifetime calculated from the experimental lifetimes and quantum yields combined with the Strickler-Berg equation, one predicts molar absorptivites (i.e. the $\varepsilon_{max}(\tilde{\nu}_{ex})$-values) that are 40% higher than the obtained experimental values. This would imply a considerable amount of impurities in the compounds, which can be excluded from analyses of the synthesised compounds [2]. Could the validity of the Strickler-Berg equation be questioned despite the mirror symmetry between absorption and fluorescence spectra? Anthracene is a well known aromatic molecule in which the lowest absorption band [$\varepsilon_{max}$ (26,700 $cm^{-1}$)=8,500 $mol^{-1}$ $dm^3 cm^{-1}$ in hexane] also exhibits a mixed polarisation [13, 15]. Moreover, the mirror symmetry is reasonably well fulfilled. The predicted radiative lifetime of anthracene is 12.9 ns, whereas that obtained from the experimental fluorescence lifetime and quantum yield is 16.6 ns [16]. Notice that the radiative lifetime, calculated from the Strickler-Berg equation is shorter than the experimental value ($\tau_0^{exp}$). Actually, $\tau_0^{exp} > \tau_0$ for fluorescent compounds showing a significant deviation between $\tau_0^{exp}$and $\tau_0$ [16, 17]. Thus, the compounds studied here show rather long radiative lifetimes and exhibit spectral mirror symmetry but the experimental radiative lifetimes are shorter than the predicted.

*Influence of oxygen quenching* Compounds **2a** and **2b** dissolved in chloroform showed no significant change upon degassing, neither in fluorescence spectral shape nor in intensity. This was performed by flushing the samples with Ar. Thus, despite the long lifetime of **2b**, oxygen is not apparently influencing the excited state lifetime.

**Table 1** The fluorescence quantum yields ($\Phi_f$) and lifetimes ($\tau_f$) of the studied compound when dissolved in non-degassed glycerol and dichloromethane at 298 K. The fluorescence reference used is described elsewhere [11]. Using these data radiative lifetimes ($\tau_0^{exp} = \tau_f/\Phi_f$) were calculated

| Compound | $\Phi_f$ (glycerol) ($\lambda_{ex}$=410nm) | $\tau_f$/ns (glycerol) ($\lambda_{ex}$=410nm) | $\tau_0^{exp}$/ns | $\Phi_f$ ($CH_2Cl_2$) ($\lambda_{ex}$=396nm) | $\tau_f$/ns ($CH_2Cl_2$) ($\lambda_{ex}$=404nm) | $\tau_0^{exp}$/ns |
|---|---|---|---|---|---|---|
| 2a | 0.06±0.01 | 1.6±0.03 | 25.3±3 | 0.03±0.01 | 1.1±0.08 | 33.0±9 |
| 2b | 0.72±0.03 | 17.0±0.05 | 23.7±3 | 0.49±0.04 | 13.4±0.05 | 27.5±3 |
| 2c | 0.46±0.05 | 11.3±0.05 | 24.9±3 | 0.29±0.01 | 8.0±0.03 | 27.7±1 |
| 2d | 0.66±0.03 | 17.1±0.04 | 26.0±1 | 0.40±0.02 | 11.2±0.02 | 28.1±2 |
| 2e | 0.56±0.04 | 14.0±0.09 | 25.1±2 | 0.20±0.04 | 5.45±0.01 | 28.4±6 |
| 2f | 0.57±0.08 | 16.2±0.05 | 29.4±4 | 0.53±0.02 | 14.1±0.04 | 26.6±1 |

## Concluding remarks

The compounds **2b–2f** differ in the sense that they contain electron withdrawing or electron donating substituents. These groups exhibit a negligible influence on absorption and fluorescence spectra of the studied 2-pyridones. There is, however, a subtle influence on intramolecular relaxation rates, which is revealed by the obtained significant changes of the fluorescence lifetimes.

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